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Pediatric Nephrology

Eighth Edition

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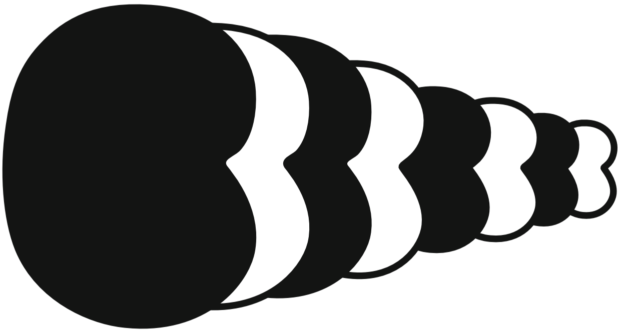
Pediatric Nephrology

Francesco Emma • Stuart L. Goldstein •
Arvind Bagga • Carlton M. Bates •
Rukshana Shroff
Editors

Pediatric Nephrology

Eighth Edition

With 339 Figures and 211 Tables



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Preface

The previous seven editions of *Pediatric Nephrology* have established this text as the reference standard for clinical care and research related to the care of the child with kidney disease. The intervening years since the last edition, as is usual, has witnessed a transformation in kidney-related care catalyzed by novel diagnostics, omics-based discoveries, and innovative medications and devices.

As Editors of this eighth edition of *Pediatric Nephrology*, we have aimed to keep up with these changes as well as the shift from hardcopy text reference to more visual and electronic educational structures. We have taken a hard look at the previous editions of *Pediatric Nephrology* and have made substantial changes to the format, scope, and goals of the textbook. Our objectives are to make *Pediatric Nephrology* more relevant to the academic pediatric nephrologist, whether they be focused on clinical care and/or research across the full spectrum of kidney disease in children. For that reason, we have coalesced many of the clinical and science chapters in a single chapter devoted to the topic at hand as well as integrated the pathology content into each relevant chapter instead of a separated chapter. We reduced the size of the textbook from three to two volumes, to make it more concise and affordable, without sacrificing the comprehensiveness of the content. We also worked with the publisher, Springer, to upgrade the illustrative material significantly. *Pediatric Nephrology* is one of Springer's Reference Series textbooks and therefore has the highest priority in their portfolio. Finally, *Pediatric Nephrology* is endorsed as the authoritative textbook in the field by The International Pediatric Nephrology Association.

Much has also changed over the past 6 years in terms of the leadership of the textbook. Drs. Avner, Harmon, Niaudet, and Yoshikowa stepped down as part of a planned editorial succession. In their stead, Drs. Bagga, Bates, and Shroff joined Drs. Emma and Goldstein to complete the editorial team. The additions of these international leaders in basic science, chronic kidney disease, and education have clearly enhanced the depth and quality of this edition.

Sadly, since the publication of the seventh edition, our community has lost Drs. Avner and Harmon. The Editors are extremely grateful to Dr. Avner for his counsel and advice during the negotiations with Springer, and our desire to take the textbook in a new direction, he was completely supportive of our ideas. Dr. Avner was going to write a preface to this edition as well, but although he did not have the opportunity, his support and impact are seen within these pages.

The last few years have certainly given us all pause and time for reflection. The Editors acknowledge all authors who met deadlines despite the whirlwind of additional stresses placed upon us all by the pandemic. Dr. Shroff dedicates this book to her mentor, Professor Lesley Rees. Lesley's contribution to the fields of CKD, dialysis, and nutrition have changed the way a generation of children were treated. She helped train pediatric nephrologists globally, always emphasizing the importance of clinical expertise, dedication to excellence, and compassion. She will be remembered for her humanity, generosity, and graciousness as much as for her many brilliant academic contributions. Dr. Bates dedicates the book to Dr. Michel Baum, a mentor during fellowship and someone who helped him in his career so many ways beyond the training years. He is a true leader in pediatric nephrology having trained so many physicians. He has also been a role model for scholarship in our specialty and has made many groundbreaking discoveries. Dr. Bates also dedicates this book to his wife, Erin, and two children, Helena and CJ, who endured many changes in address and gave him unconditional love and support. Dr. Bagga dedicates this book to all young patients with chronic kidney diseases and their families, living in under-resourced areas of the world, as they navigate their lives through innumerable challenges. These children have given us the opportunity to learn, serve, and optimize their care. Drs. Emma and Goldstein dedicate this book to the memory of their mentor, Dr. William Harmon, who was inspirational to them in so many ways. Dr. Harmon led by example first, do right by the patient and take care of the paperwork later. Furthermore, he listened to what his fellows wanted to do with their career and created the opportunities for them, instead of forcing a path on them.

We hope at whatever stage your career finds you, the eighth edition of *Pediatric Nephrology* serves your needs to provide the best care for your patients and their families and gives you foundation to improve their care in the future. They deserve nothing less.

Rome, Italy — Francesco Emma
Cincinnati, USA — Stuart L. Goldstein
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Part I

Developmental Physiology and Diseases

Pre-natal Development of the Kidneys and Urinary Tract

1

Carlton M. Bates, Jacqueline Ho, Sunder Sims-Lucas, and Kimberly Reidy

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Abstract

The mammalian kidney and lower urinary tract maintains and regulates water balance, acid–base homeostasis, electrolyte equilibrium, and waste excretion. The performance of these activities depends on the development of specific cell types in a precise temporal and spatial pattern. Defects in the process can lead to insufficient numbers of nephrons and/or dysfunctional ureters and/or bladders that fail to propel urine forward leading to secondary renal defects. Over the past several decades, considerable advances have been made in understanding the molecular basis for this developmental program. Defects in this program result in congenital anomalies of the kidney and urinary tract (CAKUT), which are leading causes of chronic kidney disease and renal failure in children. These developmental disorders range from renal malformations, such as renal aplasia (absence of the kidney), dysplasia (failure of normal renal differentiation), and hypoplasia (smaller kidneys), to urinary tract abnormalities such as hydronephrosis, vesicoureteral reflux, and duplicated collecting systems. Moreover, many of the molecules identified in animal models as critical for renal development have been validated as important in human CAKUT. This chapter describes renal embryology and molecular control of kidney and urinary tract development, as a means to understand the developmental origins of human CAKUT.

Keywords

Kidney development · Nephron induction · Ureteric branching morphogenesis · Glomerulogenesis · Renal stroma · Kidney vasculature · Bladder development · Ureter development · Urothelium · Urinary tract mesenchyme

Introduction

The mammalian kidney functions as a key regulator of water balance, acid-base homeostasis, maintenance of electrolytes, and waste excretion. The performance of these activities depends on development of specific cell types in a precise temporal and spatial pattern, to produce a sufficient number of nephrons. Over the past several decades, considerable advances have been made in understanding the molecular basis for this developmental program. Defects in this program result in congenital anomalies of the kidney and urinary tract, which are the leading causes of chronic kidney disease and renal failure in children. These developmental disorders range from renal malformations, such as renal aplasia (absence of the kidney), dysplasia (failure of normal renal differentiation), and hypoplasia (smaller kidneys), to urinary tract abnormalities such as vesicoureteral reflux and duplicated collecting systems. This chapter describes the embryology of the kidney and urinary tract, as a means to understand the developmental origins of these disorders.

Human kidney development starts in the fifth week of gestation, and new nephrons are formed until approximately 32–34 weeks gestation [1]. Remarkably, nephron endowment is quite variable ranging from 200,000 to 1.8 million nephrons per person [2]. Human kidney growth after 34 weeks gestation occurs due to maturation of existing nephrons. Since the mature mammalian kidney cannot compensate for nephron loss due to renal injury by the *de novo* generation of nephrons, the number of nephrons present at birth is an important determinant of long-term kidney health. This idea is supported by the association of reduced nephron number with hypertension and chronic kidney disease [3, 4]. Equally important to nephron endowment is the structural development of each nephron or nephron patterning.

The formation of kidneys in utero involves different critical developmental processes. Differentiation is the process by which precursor cells or tissues mature into more specialized cells. For example, early renal mesenchymal cells have the potential to differentiate into nephron epithelia or stromal cells. Morphogenesis describes the process whereby cells and tissues acquire three-dimensional patterns. This is particularly important in the kidney, as the three-dimensional relationship between the nephrons, vasculature, and collecting system are critical for normal kidney function. Finally, the regulation of cell number at different stages of development is crucial, involving a balance between proliferation and programmed cell death or apoptosis. All of these processes are tightly regulated spatially and temporally during kidney development.

Genetic, epigenetic, and environmental factors all regulate differentiation, morphogenesis, and cell number within the developing kidney [5–7]. Mutational analyses in animal models have provided significant insights into the genetic control of renal development. Genes critical for kidney development in animal models include transcription factors that act as master regulators of other genes, growth factors that signal to other cells, and adhesion molecules that regulate how cells interact with each other and with the extracellular matrix. Increasingly, analyses of humans with congenital renal malformations (such as renal aplasia or duplex kidneys) have frequently identified gene mutations originally described in animal models [8]. Recent studies have also implicated epigenetic mechanisms (defined as heritable changes in gene activity that are not caused by changes in DNA sequence) in regulating nephron formation. These mechanisms include DNA methylation, chromatin remodeling, and non-coding regulatory RNAs [9, 10]. Finally, environmental influences that interact with genetic and epigenetic factors are also important in determining nephron number and patterning. For example, Vitamin A deficiency leads to decreased nephron number in rodents, and has been implicated in renal size in humans [11, 12].

Studying Kidney and Urinary Tract Development

The methods for studying the molecular and genetic control of kidney development have continued to evolve over the past several decades. Visualization of tissue morphology and expression of individual genes and proteins in the developing kidney have traditionally been performed on tissue sections by general staining (e.g., hematoxylin and eosin), detecting messenger RNA (mRNA) via in situ hybridization, or protein via immunohistochemistry (Fig. 1a–c). Technologies to assay the transcriptome of the developing kidney and/or different kidney tissue compartments in a genome-wide, unbiased fashion have advanced significantly in the last decade to encompass high-throughput RNA sequencing, small RNA sequencing and single-cell RNA sequencing (which enables the measurement and analysis of the transcriptome of individual cells within a tissue). These techniques have informed large public databases that describe the gene expression of the developing kidney, including the GenitoUrinary Molecular Anatomy Project, (www.gudmap.org) and Eurexpress (www.eurexpress.org) [13, 14]. These next-generation sequencing approaches are now widely used to identify DNA mutations in the genome (whole exome and whole genome sequencing), changes in chromatin accessibility (assay for transposase-accessible chromatin using sequencing, ATAC-seq), and protein interactions with chromatin (chromatin immunoprecipitation then DNA sequencing, ChIP-seq). Moreover, with the decreasing cost associated with next-generation sequencing, this technology has moved into clinical practice with whole exome and whole genome sequencing often being used to identify mutations associated with kidney disease in patients [15].

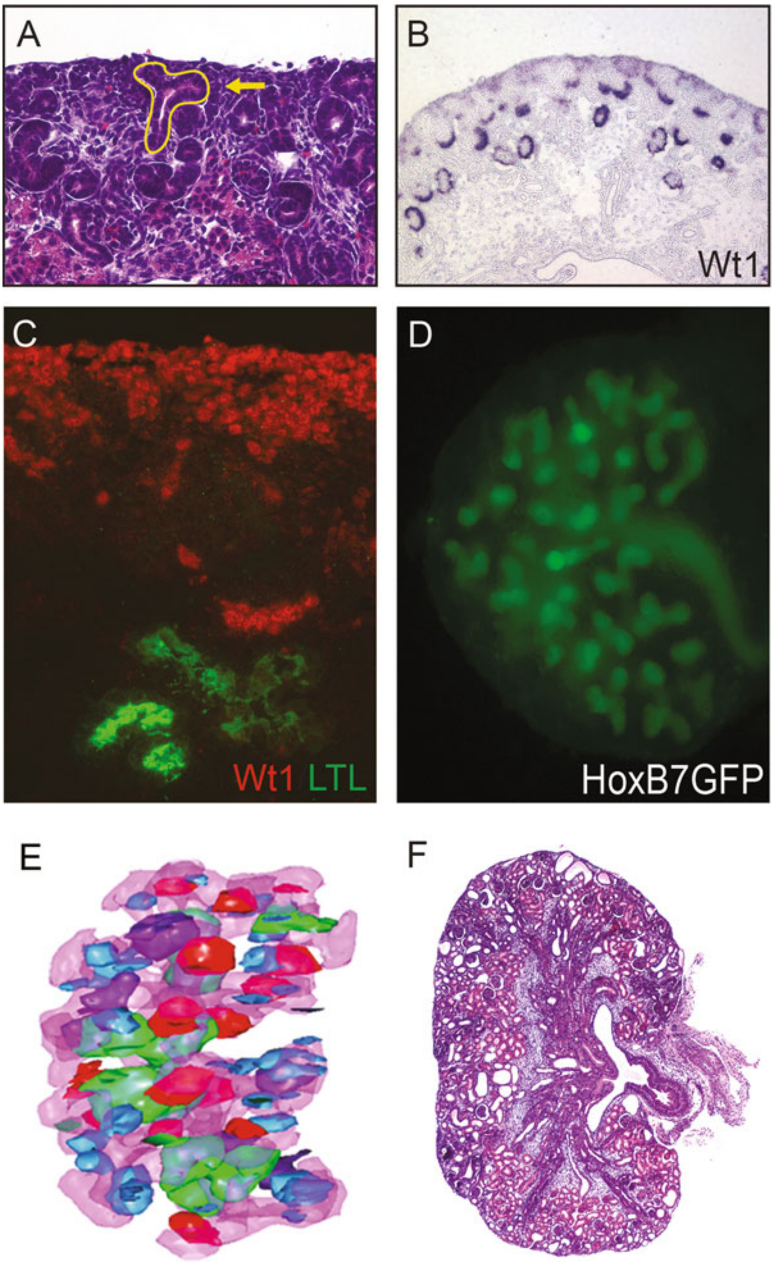

Fig. 1 **Experimental methods utilized to study kidney development**. (**a**) Hematoxylin and eosin (H&E) stained tissue section of a control postnatal day 0 mouse kidney. The ureteric bud is outlined in yellow, arrow points to the cap metanephric mesenchyme. (**b**) In situ hybridization in a control mouse embryonic day 16 tissue section for the transcription factor, Wt1, which stains the metanephric mesenchyme and developing glomeruli. (**c**) Immunofluorescent staining in a control embryonic day 14 mouse tissue section for the transcription factor, Wt1 (red), and lotus tetragonolobus lectin (LTL, green), which stains the proximal tubule. (**d**) Embryonic culture of a transgenic embryonic day 11 HoxB7GFP mouse kidney, demonstrating branching ureteric structures (green) after 5 days of growth. (**e**) 3D reconstruction of an embryonic day 13 mouse kidney with the ureteric epithelium depicted in pink and the developing nephron types including: renal vesicles (blue), comma-shaped bodies (red), S-shaped bodies (purple) and glomeruli (green). (Reproduced with kind permission from Springer Science+Business Media. Sims-Lucas [184], Figure 3F). (**f**) Hematoxylin and eosin stained tissue section of a postnatal day 0 mouse kidney lacking microRNAs in the ureteric lineage using a conditional knockout approach (*HoxB7Cre; Dicer*$^{flx/flx}$)

A classical technique to analyze kidney development is to culture rodent embryonic kidneys *in vitro* as explants. Studies using these methods were the first to show that reciprocal interactions between the metanephric mesenchyme and the ureteric bud are critical to induce the formation of new nephrons and ureteric branching (Fig. 1d) [16]. Moreover, kidney explants allow one to modulate the expression and function of specific genes and proteins using reagents such as antisense oligonucleotides or blocking antibodies. While these experiments have been illuminating, the growth of embryonic kidney explants differs from kidney development *in vivo* in several key ways: lack of blood flow, growth limitations from diffusion of the culture media across the air-media interface, and distortions in the three-dimensional kidney architecture as explants flatten in culture. More recently, efficient and reliable protocols to direct the differentiation of human induced pluripotent stem cells into three dimensional kidney organoids have been developed using insights derived from the study of kidney development [17]. Kidney organoids have emerged as a useful system to study the pathophysiology of diseases like polycystic kidney disease, to understand drug toxicities, and to inform efforts directed towards high-throughput drug screening and renal regeneration. Kidney organoids will be discussed in detail in ▶ Chap. 4, "Stem Cells and Kidney Regeneration."

Because kidney function is critically dependent on the complex three-dimensional structure of the nephron and the number of nephrons that are generated, several methods have been developed to generate more physiological and quantifiable three-dimensional reconstructions of developing kidneys and urinary tracts. One method utilizes serial sectioning through developing kidneys, histological staining, projection of each serial image onto a monitor to identify each tissue lineage, and rendering of the serial images into a three-dimensional image (Fig. 1e) [18]. This technique allows for the quantification of both developing nephron structures and the branching ureteric tree. Optical Projection Tomography can image through the full thickness of a developing kidney that has been whole mount stained for specific cells and permits the quantification of these elements [19]. Advances in imaging techniques (such as novel tissue clearing protocols, light sheet microscopy, and high-resolution confocal microscopy), along with the capability to analyze large image datasets, now provide us with the ability to image fluorescent markers in the developing kidney at higher spatiotemporal resolution in ever-larger, intact samples without phototoxicity [19]. Similarly, advances in magnetic resonance imaging tools offer the potential to count nephron number and analyze local tubular function experimentally, with potential to impact on clinical practice [20].

Physical, chemical, and genetic strategies can be used to manipulate developing kidneys *in vivo*. For example, ureteric obstruction in utero in sheep and monkeys results in hydronephrotic kidneys with renal dysplasia [21, 22]. In addition, dietary manipulations including high doses of vitamin A or dietary protein restriction result in kidney and urinary tract defects [23]. Transgenic approaches have also been used to drive gene expression in specific spatiotemporal patterns, usually in the mouse. In these experiments, a transgenic construct consisting of a tissue-specific promoter and the gene of interest is randomly inserted into genome, leading to expression of that gene in a tissue specific pattern [24]. The limitations of this approach include: (1) that the random insertion can result in unintended changes in gene expression (due to other nearby promoters/enhancers near the site of integration); (2) that the insertion of the transgene into the genome may lead to loss of function of an endogenous gene and; (3) epigenetic factors may silence the construct. The increased utilization of Bacterial artificial chromosome (BAC) constructs, which contain more endogenous promoter elements than are found in traditional plasmid constructs, has led to more faithful and reliable transgene expression.

As opposed to transgenic approaches, homologous recombination is the method whereby a gene is "knocked-out of" or "knocked-into" the mouse genome [24]. Using these methods, a gene of interest is deleted so that it becomes non-functional or an exogenous gene (such as a green fluorescent reporter) is added to the genome at a specific locus. A limitation to traditional knockout techniques are that global loss of function of the gene may result in extra-renal effects (such as early embryonic lethality), which can impact or severely limit the study of the gene's function in the kidney. Given these limitations, it has become more common to perform conditional gene targeting (e.g., with the Cre-/loxP system) (Fig. 1f) and/or inducible gene targeting (e.g., with tamoxifen), allowing for kidney and/or urinary tract specific gene deletion (using a kidney specific Cre-) and/or at a particular time (driving induction of gene targeting with a drug).

While most investigators using genetically modified animals utilize mice, a growing number of scientists study kidney development in other model systems, such as avians, zebrafish, and Xenopus [25]. Advantages of these systems include the ability to produce larger number of embryos in a shorter period of time than in mammals. While many of these models, such as zebrafish and Xenopus usually focus on the pronephros, many of the genes that pattern this "simpler" kidney are required in mammalian metanephric kidneys. Finally, the advent of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (Cas) techniques now allow for relatively quick and easy genetic modifications of virtually any cell or animal model desired [26].

Origin of the Mammalian Kidney

The mesoderm forms as one of the three embryonic germ layers during gastrulation. The mammalian kidney develops from the intermediate mesoderm, lying between the paraxial and lateral plate mesoderm, on the posterior abdominal wall of the developing embryo. In mammals, three pairs of embryonic kidneys develop from the intermediate mesoderm: the pronephros, the mesonephros and the metanephros (Fig. 2) [5, 27]. At the height of their development, the pronephros and mesonephros extend from the cervical to the lumbar levels of the developing embryo. The pronephric and mesonephric nephrons are induced to differentiate by signals from the adjacent nephric ducts, paired epithelial tubules running in a longitudinal course along the embryo on either side of the midline. The nephric duct (also known as the Wolffian duct) continues to grow caudally in the embryos to eventually fuse with the cloaca, which ultimately gives rise to the bladder (see below). The specification of the nephric duct is dependent on the expression of several transcription factors, such as *Paired box 2 (Pax2)*, *Pax8,* and *Gata binding protein 3* (*Gata3*). The pronephros is not functional in mammals, but is the functional kidney in larval fish and frogs. The mesonephros becomes the mature kidney in these lower species and is functional in mammals during embryogenesis. Ultimately, the pronephros and mesonephros largely degenerate in mammals, although remnants of the mesonephros persist in the mammalian male reproductive organs including the rete testis, efferent ducts, epididymis, vas deferens, seminal vesicle, and prostate.

The mature mammalian kidney, the metanephros, is derived from two main tissues, the ureteric bud and metanephric mesenchyme [6, 27]. Starting at approximately embryonic day 10.5 in the mouse and the fifth week of gestation in humans, reciprocal inductive signals cause the ureteric bud to evaginate from the caudal portion of the nephric duct, and the metanephric mesenchyme to condense around the ureteric bud ampulla and tips to form nephron progenitors (Fig. 2). The specification of the metanephric mesenchyme from the posterior intermediate mesoderm requires several transcription factors, including *Odd-skipped related 1* (*Osr1*) [28] and *Lim homeobox 1* (*Lhx1*). The ureteric bud ultimately gives rise to the collecting system, including the collecting ducts, renal calyces, renal pelvis, and ureters [1, 5]. In turn, the nephrogenic metanephric mesenchyme (nephron progenitors derived from the metanephric mesenchyme) differentiates into the epithelial cells that comprise the mature nephron, including the parietal cells and podocytes of the glomerulus, proximal convoluted tubule, the ascending and descending limbs of the loops of Henle, and the distal convoluted tubule [1, 5]. Just after the condensation of the nephrogenic mesenchyme around the initial ureteric bud, a third renal lineage, the stromal metanephric mesenchyme (or renal stroma), appears peripheral to the nephrogenic mesenchyme. The renal stroma

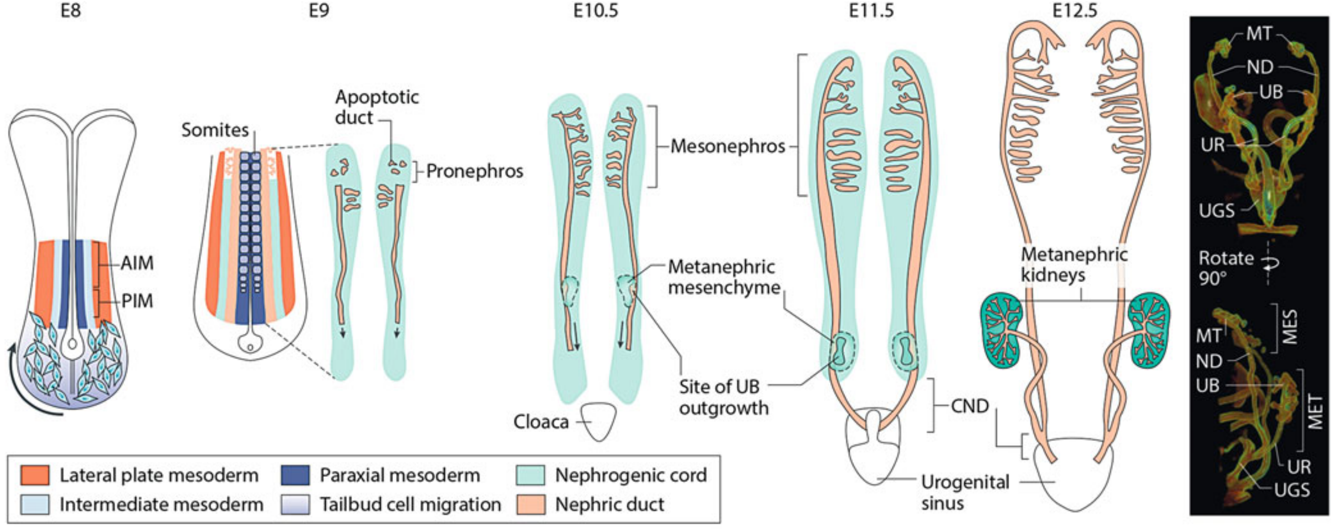
E8
E9
E10.5
E11.5
E12.5
AIM
PIM
Somites
Apoptotic duct
Pronephros
Mesonephros
Metanephric mesenchyme
Site of UB outgrowth
Cloaca
CND
Urogenital sinus
Metanephric kidneys
MT
ND
UB
UR
UGS
Rotate 90°
MES
MET
Lateral plate mesoderm
Intermediate mesoderm
Paraxial mesoderm
Tailbud cell migration
Nephrogenic cord
Nephric duct

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Fig. 2 **Overview of mouse kidney development.** The mammalian kidney originates from the band of intermediate mesoderm sandwiched between the lateral plate and paraxial mesoderm at embryonic day (E) 8.0 in mice. Around E9.0, a population of cells from the anterior intermediate mesoderm (AIM) gives rise to the pronephric duct rostrally and elongates caudally to form the nephric duct (ND). By E10.5 in mice, the ND has migrated close to the cloaca and the rostral and caudal nephric tubules are generated from the AIM and posterior intermediate mesoderm (PIM), respectively. At this stage, the pronephros has undergone apoptosis, the ND gives rise to the ureteric bud (UB) and the metanephric mesenchyme arises from PIM cells present in the nephrogenic cord. At E11.5, the UB, which later elaborates into the entire ureteric collecting duct system, starts dividing with a simple initial 'T-shaped' bifurcation. At this stage, remodeling of the common nephric duct (CND) begins and will ultimately result in the connection of the ureters with the developing urogenital sinus (UGS)/bladder. Around E12.5, metanephric kidney development rapidly progresses, the nephric tubules (MT) are positioned rostrally and connected to the ND, and all components are connected to the UGS through the developing ureter (UR) and the CND. The right-hand panel shows the projection of an optical tomography dataset of CADHERIN-1 expression at E12.0. MES, mesonephros; MET, metanephros. (Reprinted by permission from Springer Nature. Short and Smyth [27], adapted from Figure 1)

develops into perivascular cells, vascular smooth muscle, fibroblasts, mesangial cells, rennin cells, and even some peritubular endothelial cells (see below).

Many key signaling pathways have been shown to drive reciprocal interactions between the ureteric epithelium, the renal mesenchymal lineages, and the renal vasculature [7]. Ureteric bud outgrowth depends on inductive signals from nephron progenitors, stromal cells and angioblasts [6], as well as from itself [29]. The nephrogenic mesenchyme relies in part on signaling from the ureteric bud and renal stroma for self-renewal and well as for initiation of nephron formation [7]. Subsequent nephrogenesis (i.e., patterning and differentiation of nephron epithelia) is highly dependent on factors from both ureteric epithelial and stromal cells.

Nephron Formation

As noted, the metanephric mesenchyme gives rise to nephrogenic mesenchyme or nephron progenitors, which self-renew and have the capacity to form the multiple epithelial cell types of the nephron. Much research has focused this progenitor cell population, which is critical for determining nephron endowment and thus long-term kidney health.

Anatomically, there are several steps that take place in nephron formation [5, 30, 31] (Fig. 3). After the ureteric bud has penetrated the metanephric mesenchyme, nephron progenitors condense around the first ureteric ampulla, forming "cap mesenchyme." As the ureteric bud continues to branch and elongate, the nephrogenic mesenchyme continues to form new caps surrounding each ureteric tip. After the initial few ureteric branches, the earliest cap mesenchymal cells receive spatiotemporal cues to begin the differentiation process to form epithelialized renal vesicles. Subsequent growth and differentiation of the renal vesicle results in formation of the comma-shaped body, which then lengthens to form the S-shaped body. The lower limb of the S-shaped body begins to differentiate into glomerular podocytes. During this time, endothelial cells migrate into the cleft of the lower limb of the S-shaped body and will ultimately form the glomerular capillary loops. Simultaneously, nascent mesangial cells, derived from renal stroma (see below) also migrate into this cleft. Thus, the lower limb of the S-shaped body ultimately forms the glomerulus. Concurrently, the middle and upper limbs of the S-shaped body elongate and differentiate into nephron tubules including proximal tubules, loops of Henle (including descending and ascending limbs) and distal convoluted tubules. The terminal ends of the distal convoluted tubules eventually connect to ureteric epithelia, which ultimately form collecting system (collecting ducts, renal pelvis, and ureters). Nephrogenesis repeats in a radial fashion with

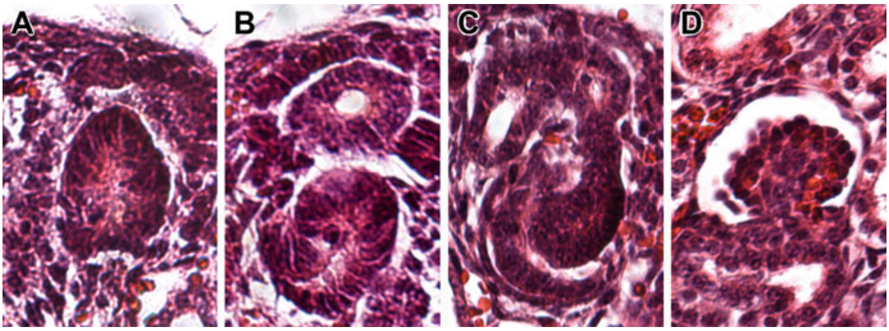

Fig. 3 H&E stained sections showing the four stages of nephron formation in mice. (**a**) Image of a renal vesicle, the first stage of nephron formation. (**b**) Image of a comma-shaped body that differentiated from a vesicle. (**c**) Image of an S-shaped body, the third stage of nephron formation. (**d**) Image of an immature glomerulus that differentiated from the lower limb of the S-shaped body. (Reproduced with kind permission from Springer Science+Business Media: Sims-Lucas [18], Figure 3, panels A–D)

the first nephrons forming in the juxtamedullary regions and last in the peripheral cortex, until the full complement of nephrons is reached.

During prenatal and/or postnatal life, each nephron increases in size and complexity as it matures. Starting in the first month of life, maturing proximal tubules transition from a columnar to cuboidal epithelium, develop microvilli, and increase their tubular dimensions. While the earliest limbs of the Henle loop are located in the renal cortex, subsequent maturation and elongation of these limbs in utero results in the loops pushing through the cortico-medullary boundary in term infants. Postnatal maturation results in the Henle loops eventually reaching the inner renal medulla in the mature kidney. Thus, the urinary concentrating capacity of newborn infants is limited by a reduced medullary tonicity gradient, due to the relatively shorter loops of Henle. Finally, as the distal convoluted tubule matures a portion of the cells are found in close proximity to the future vascular pole of the developing glomerulus, where they develop into the macula densa.

Specification of Nephron Progenitors/Cap Mesenchyme

Differentiation of the intermediate mesoderm and metanephric mesenchyme into nephron progenitors and their derivatives is genetically defined by the sequential upregulation of several transcription factors, cell adhesion molecules, and growth factors [32]. The intermediate mesoderm and early metanephric mesenchyme express the transcription factors *Sal-like 1* (*Sall1*), *Sine oculis homeobox homolog 1* (*Six1*), *Eyes absent homolog 1* (*Eya1*), and the secreted peptide growth factor, *Glial-derived neurotrophic factor* (*Gdnf*). Induction of cap mesenchyme/nephron progenitors by the ureteric bud tips [32–35] is marked by expression of transcription factors such as *Wilms tumor 1* (*Wt1*), *Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain, 1* (*Cited1*) and *Sine oculis homeobox homolog 2* (*Six2*), as well as the transmembrane molecules *cadherin-11* and *α8 integrin*. The earliest epithelial derivative of the cap mesenchyme, the renal vesicle, is marked by the transcription factors *Pax2* and *Lhx1*. In recent years, elegant studies have focused on spatiotemporal expression of these factors in the mouse and human developing kidney comparing the similarities and variabilities between these two species [36]. In addition, high resolution imaging has been used to define the anatomical arrangement of the developing nephrons in mouse compared to humans.

The use of tissue specific gene knockouts generally via conditional transgenic techniques has revealed the importance of several transcription factors (including many mentioned above) in the specification of the cap mesenchyme. Conditional homozygous deletion of *Eya1*, *Six1*, *Pax2*, *Wt1*, *Sall1*, *Six2* or *Lhx1* leads to bilateral renal aplasia or severe renal dysgenesis from defects in cap mesenchyme specification and/or differentiation [36]; these mesenchymal defects are often accompanied by ureteric induction and/or branching abnormalities due in large part due to loss of GDNF signaling from the metanephric mesenchyme. *Pax2* mutant mice generate a metanephric mesenchyme that is unable to differentiate into nephrons and fail to form the nephric duct, which is required for ureteric bud induction. In *Lhx1* and *Sall1* mutants, the metanephric mesenchyme does induce ureteric bud formation, but it fails to elongate and branch and the mesenchyme is once again unable to differentiate into nephrons. In *Wt1* mutants, a defective metanephric mesenchyme forms, but rapidly undergoes apoptosis. SIX2, a specific marker of nephron progenitors, is required for maintenance of progenitor cells, but not for nephron differentiation; deletion of *Six2* in mice results in the formation of ectopic nephron tubules and the rapid depletion of nephron progenitor cells. Similarly, the p53-E3 ubiquitin ligase, Murine double minute 2 (MDM2) appears critical for maintenance of nephron progenitor cells [37, 38].

Recent studies have identified factors that govern the delicate balance of self-renewal and differentiation of nephron progenitors, including WNT genes. Studies from the mid-1990s showed that Lithium chloride, a potent inducer of Wnt signaling, was able to drive tubulogenesis in isolated rodent metanephric mesenchyme cultures

[39–41]. More recently WNT9b, secreted from ureteric bud cells, was shown to be required for the differentiation of nephron progenitor cells [42]. Genetic deletion of *Wnt9b* resulted in a failure of nephron progenitors to undergo the mesenchymal to epithelial transition that is required to form the renal vesicle. A subsequent study revealed that *Wnt9b*, in concert with signals from the renal stroma, plays an essential role in mediating the decision of nephron progenitors to self-renew or differentiate.

Another major signaling pathway that has been shown to mediate nephron progenitors survival is the Fibroblast growth factor (FGF) signaling pathway [42]. FGF ligands are secreted peptides that bind and signal through their receptor tyrosine kinases, FGF receptors (FGFRs). Isolated nephrogenic zone cell culture studies revealed that addition of FGF1, 2, 9, and 20 ligands drive expression of nephron progenitor markers and progenitor proliferation. Furthermore, *in vivo* mouse studies showed that *Fgf9* and *Fgf20* are critical for maintaining nephron progenitor survival, proliferation, and competence to respond to inductive signals. FGF20 mutations in humans were also shown to be associated with severe renal dysplasia. Other work in mice has identified that the FGFRs critical for metanephric mesenchyme development are *Fgfr1* and *Fgfr2*. Combined conditional deletion of *Fgfr1* and *Fgfr2* in the metanephric mesenchyme leads to severe renal dysgenesis.

Several studies have shown that a balance between cell survival and apoptosis is necessary for normal nephron progenitor function. Classic *in vitro* studies using isolated metanephric mesenchyme have shown that nephron progenitors undergo significant apoptosis when cultured without an inducer [43]. Co-culture with isolated ureteric buds or heterologous inducers, such as embryonic spinal cords, dampens apoptosis and drives progenitor survival [5, 44]. Other studies have identified several factors that when added to metanephric mesenchyme or nephrogenic zone cell cultures drive progenitor survival, including Transforming growth factor-β2 (TGF-β2), TGFα, Leukemia inhibitory factor (LIF), Epidermal growth factor (EGF), FGF2, and Bone morphogenetic protein 7 (BMP7) [45–48]. Whether some or all of these factors act as endogenous nephron progenitor inducers remains to be determined. Counterbalancing cell survival, apoptosis is required for normal nephron progenitor function. Moreover, suppression of apoptosis via pharmaceutical or genetic means leads to kidney malformations including abnormal ureteric branching and defective nephrogenesis [49, 50]. Relative "over-abundance" of nephron progenitors also leads to renal epithelial and/or stromal cell defects [51, 52].

Much focus over the last decade has been on understanding what drives nephron progenitors to self-renew versus differentiate. One group found that FGF9 and a potent WNT agonist (CHIR 99021) drove isolated nephron progenitors to renew (instead of differentiating) [33, 53] (see ▶ Chap. 4, "Stem Cells and Kidney Regeneration" for a more in depth discussion of this subject). Another seminal study found that the relative age of a nephron progenitor highly influenced decisions to renew or differentiate [54]. Transplantation of "young" and "old" nephron progenitors into young nephron niche revealed that the older progenitors spent far less time in the undifferentiated state. Thus, it appeared that older progenitors had been imprinted and were more committed to differentiation than the younger progenitors. Other studies have focused on the role of metabolism in governing nephron progenitor decisions to renew or differentiate. One found that p53 (a tumor suppressor) stimulated glycolysis in nephron progenitors, which drove proliferation and self-renewal. Moreover, forcing a switch to aerobic oxidative phosphorylation was sufficient to drive nephron progenitor differentiation [55]. Another study showed nephron progenitor renewal also depended on a relative physiological hypoxia that drives glycolysis and renewal. Moreover, prolonging/exacerbating hypoxia (by lowering oxygen tension or by genetically driving hypoxia inducible factors) forced persistent glycolysis and decreased ability of nephron progenitors to differentiate, all of which could be rescued by using a glycolytic inhibitor [56, 57].

Epigenetic factors also regulate nephron progenitor specification, survival, and potential for differentiation. Histone deacetylases (HDACs) are enzymes that remove acetyl groups from histones, which then modulate (usually stimulate) gene transcription. Class I HDACs are highly expressed in nephron progenitors and are required for proper expression of several key developmental genes including *Osr1*, *Eya1*, *Pax2*, *Wt1*, and *Wnt9b* (among others) [58]. MicroRNAs (miRNAs) are small non-coding RNAs that bind to specific mRNA targets to block translation and promote mRNA degradation. Conditional targeting of *Dicer*, an enzyme required for processing of all miRNAs, in mouse nephron progenitors, led to a loss of the progenitors due to excessive apoptosis, likely from upregulation of the pro-apoptotic protein, BIM. Another study revealed that conditional deletion of a specific miRNA cluster, the miR-17 ~ 92 complex, in nephron progenitors led to a decreases in progenitor proliferation, fewer numbers of nephrons, proteinuria, and podocyte damage. Moreover, these reports was the first to identify a specific miRNA cluster essential for kidney development [59, 60].

Nephron Induction

The initial differentiation step of nephron progenitors is to undergo a mesenchymal to epithelial transition to form the renal vesicle. *In vitro* experiments utilizing isolated rodent metanephric mesenchymal rudiments (similar to and including some of the survival studies noted above) have identified exogenous factors that stimulate nephron progenitors to undergo tubulo-epithelial differentiation [44]. Some of these growth factors can act alone or in concert with others and include FGF2, LIF, TGFβ2, growth/differentiation factor-11 (GDF-11), and WNT1/4. Sequestration of WNT ligands in intact rodent kidney explants by addition of secreted Frizzled-related proteins (sFRPs) led to decreases in mesenchyme-derived tubulogenesis [61]. Mouse genetic experiments have identified at least some of the critical endogenous pathways and growth factors necessary for induction of the mesenchymal to epithelial transition. For example, global deletion of *Wnt4*, normally expressed in renal vesicles, did not perturb cap mesenchyme formation; however, the mutant nephron progenitors were completely unable to form renal vesicles [62]. Conditional deletion of *Fgf8* in the metanephric mesenchyme leads to a block of nephrogenesis beyond the renal vesicle stage, possibly by interacting with Wnt4 to drive of *Lhx1* expression [63, 64]. Interestingly, global deletion of *Fgfr-like 1*, a membrane bound FGF receptor that lacks an intracellular tyrosine kinase domain, also leads to a block in nephrogenesis similar to *Fgf8* conditional mutants [65].

Nephron Segmentation

Establishment of a proper proximal-distal axis is critical for normal nephron segmentation. Reciprocally, repressive interactions between WT1 and PAX2 at early stages of nephron development appear vital to proximal-distal axis patterning [42, 66–68]. In the S-shaped body, WT1 is localized to the lower limb and inhibits PAX2 expression, which together drives cells toward podocyte fates. Transgenic mice with overexpression-expression of PAX2 throughout the embryo including embryonic nephrons develop glomerular defects and renal cystic dysplasia [69]. In contrast, PAX2 is expressed in the upper limb of the S-shaped body and represses WT1 expression, stimulating these cells to become tubular nephron segments.

Two other transcription factors critical for proximal/distal axis patterning of the nephron include *Lhx1* and *Brain specific homeobox 1* (*Brn1)*, both of which are expressed at the renal vesicle stage. Conditional deletion of *Lhx1* throughout the metanephric mesenchyme blocks nephrogenesis at the renal vesicle stage and also leads to a loss of *Brn1* expression [70]. Conditional targeting of *Brn1* in the metanephric mesenchyme does not block proximal nephrogenesis; however, the loop of Henle fails to form and distal convoluted tubules fail to terminally differentiate [71]. These results suggest that LHX1 acts earlier in nephron patterning than BRN1, which is critical distal nephron patterning.

NOTCH receptor signaling, mediated largely by *Recombining binding protein suppressor of hairless (Rbpsuh),* appears critical for proximal nephron patterning. Use of a NOTCH inhibitor in mouse metanephric kidney explants led to a loss of proximal cell fates, including glomeruli and proximal tubules. *In vivo*, conditional deletion of *Notch2* or *Rbpsuh* in mouse metanephric mesenchyme leads to an absence of proximal tubules and glomerular epithelium. Finally, ectopic NOTCH expression in nephron progenitor cells results in the premature differentiation of the progenitors into proximal nephron epithelia [72, 73].

Glomerulogenesis

The first glomeruli form during the nineth week of human gestation. Glomerular capillaries are supported by the mesangium, and the mature glomerular filtration barrier is composed of podocytes with foot processes linked by foot processes, the glomerular basement membrane, and fenestrated glomerular endothelial cells. As discussed above, the glomerular visceral epithelial cell (the podocyte) and parietal epithelial cell originate from cells at the tip of the S-Shape body (the cells furthest from the ureteric bud) (Fig. 3). Vascular endothelial growth factor (VEGF) drives endothelial cell precursors to invade the vascular cleft of the S-shape body. As discussed in more detail below, both angiogenesis (endothelial cell progenitors in the kidney) and vasculogenesis (invasion of endothelial cell precursors from outside the kidney) likely contribute to these endothelial cell precursors, which form the glomerular capillaries. The location of endothelial cell invasion determines the vascular pole of the developing glomerulus. Platelet derived growth factor (PDGF) and its receptor regulate stromally derived mesangial cell progenitors that eventually form the glomerular mesangium.

Podocytes and endothelial cells both secrete the laminins and collagens that form the glomerular basement membrane (GBM). During embryonic development, the glomerular basement membrane composition changes from laminin-1 to laminin-11 and from $\alpha-1$ and $\alpha-2$ type IV collagen chains to $\alpha-3$, $\alpha-4$, and $\alpha-5$ type IV collagen chains. The normal GBM thickness also varies with age, at about 150 nm at birth, 200 nm at one year of age, and 320–370 nm (adult thickness) by age 11 [74].

Nephron and Podocyte Endowment

Since glomeruli form near the tips of ureteric buds, the extent of ureteric bud branching influences nephron and glomerular number. Glomeruli continue to form until 32–34 weeks gestation in humans, whereas glomerulogenesis persists in mice and rats for several days after birth [1]. In newborn humans, the superficial glomeruli are the most immature and are smaller than the deeper juxtamedullary glomeruli [75]. As discussed in ▶ Chap. 2, "Postnatal Renal Maturation," the deeper glomeruli are the first to filter, and increased blood flow to the superficial glomeruli contributes to the increase in glomerular filtration rate after birth. While no new glomeruli are formed after birth, they continue to grow and mature postnatally, reaching their adult size at approximately three and a half years of age [75].

Two thirds of nephrons are formed in the last trimester, and preterm birth may have unique effects on glomerular development. An autopsy study of preterm neonates found that nephrogenesis continues after birth but ceases prematurely; the authors observed abnormal glomeruli with dilated Bowman's spaces and shrunken tufts, suggesting that *ex utero* development may disrupt glomerular differentiation [76]. In addition to nephron number, podocyte endowment may be altered by preterm birth [77]. Preterm birth is associated with higher rates of CKD in children and adults [78]. Focal segmental glomerulosclerosis has been described in children and adults born preterm, suggesting preterm birth may lead to a podocytopathy. Interestingly, children with FSGS and/or CKD associated with *APOL1* high risk gene variant had a high rate of preterm birth [79].

Podocyte Terminal Differentiation

To become podocytes, S-shape cells undergo a marked shape change from columnar epithelial cells to the octopus like shape of the mature podocyte. This shape change is accompanied by alteration in cell adhesion, with apical cell junctions migrating down the lateral aspect of the cells to reestablish cell-cell junctions at the base, the site of the slit diaphragm. The process is guided by Partitioning defective (Par) polarity proteins, which establish cell domains in many types of epithelia. The complex of PAR3/PAR6/aPKC migrates with cell junction from apical to basolateral aspects of the immature podocyte. PAR3 binds to nephrin, establishing the location of the slit diaphragm. Mice lacking podocyte *Par3*, *Par6,* or *aPKC* fail to form podocyte foot processes [80].

Other transcription factors and epigenetic factors, including microRNAs, have also been shown to be critical for podocyte differentiation. Examples of essential transcription factors include *Wt1, podocyte expressed 1* (*Pod1*), *Lim homeobox 1b (Lmx1b)*, and *Mafb*. Several studies utilizing murine genetic knockout models of *Wt1* have demonstrated its critical roles in mediating podocyte differentiation [81–84]. In humans, *WT1* mutations can lead to diffuse mesangial sclerosis, characterized by podocyte differentiation defects resulting in varied glomerular lesions and proteinuria, and can occur as an isolated disease or in association with Denys-Drash or Frasier Syndromes [85–88]. Deletion of *Pod1,* expressed in stromal cells, leads to non-autonomous podocyte defects at the capillary loop stage in mice [89]. Genetic deletion of *Lmx1b* or *Mafb* leads to podocyte differentiation defects past the capillary loop stage [90, 91]. Furthermore, mutations in the *LMX1b* gene in humans leads to Nail-Patella Syndrome, which is often associated with glomerular basement membrane thickening and proteinuria that can progress to chronic kidney disease [92, 93]. Three separate studies showed the importance of microRNAs in maintaining differentiated podocytes in the mouse. Targeted ablation of *Dicer* in murine podocytes, resulting in a loss of all miRNAs, led to podocyte injury, severe proteinuria, and tubular damage starting two weeks after birth [94–96].

Glomerular Capillary Tuft Development

Several signaling cascades have been implicated in the homing and maturation of the endothelial and mesangial precursors to form the glomerular capillary tuft. Vascular endothelial growth factor (VEGF), which is secreted from the podocytes at the S-shaped body stage, promotes recruitment of endothelial precursors to the vascular cleft [97, 98]. A balance of VEGF and a neural guidance factor Semaphorin3a that opposes VEGF is also required for normal glomerular endothelial number [99]. Angiopoietin-1 and -2, growth factors expressed by podocytes and mesangial cells, respectively, are also critical for normal glomerular capillary development [100]. Mesangial cell recruitment into the cleft is largely mediated by the secretion of platelet-derived growth factor (PDGF)–B by endothelial cells, which binds PDGF receptor-β (PDGFRβ) on the mesangial cell progenitors [101]. Mice that lack either *Pdgfβ* or *Pdgfrβ* fail to form glomerular capillary tufts demonstrating the importance of mesangial cell recruitment [102, 103]. Finally, NOTCH2 and its ligand Jagged1 are critical for glomerular endothelial and mesangial cell development. *Notch2* hypomorphic mice and *Notch2/Jagged1* compound heterozygous mice develop glomerular aneurysms and possess no mesangial cells [104].

At the S-shaped body stage, immature podocytes along the lower limb are highly proliferative and have a columnar shape with apical cell attachments and a single-layer basement membrane [105]. Concurrently, endothelial and mesangial cell progenitors are recruited into the lower cleft of the S-shaped body, which will become the vascular pole [106]. While mesangial cells originate from the renal stroma (see below), the developmental origin of the glomerular endothelium is still unclear. Transplantation of avascular rodent embryonic kidney rudiments under neonatal kidney capsule led to formation of endothelial precursors, or angioblasts originating from the graft metanephric mesenchyme [106–108]. However, engraftment of embryonic rat kidney rudiments onto avian chorioallantoic membrane led to vascular ingrowth of avian vessels into the rat glomeruli [109]. As will be expanded on in the vascular development section below, it is likely that both processes/sources contribute to formation of glomerular capillaries.

As the S-shaped body matures, the lower cleft transforms into a cup shape configuration. At this time the podocytes lose their proliferative ability [110] and differentiate, forming foot processes and slit diaphragms, specialized intracellular junctions critical for proper glomerular filtration [111, 112]. Concurrently, the composition of the glomerular basement membrane changes from laminin-1 to laminin-11, and from α −1 and α −2 type IV collagen chains to α −3, α −4 and α −5 type IV collagen chains [113]. Several mouse knockout mice models have shown how failure in these transitions lead to structural and functional glomerular basement membrane defects [114–116]. At this stage of development, the nascent mesangial cells act as a scaffold for the formation of the glomerular capillary loops and ultimately form the supportive core for the entire glomerulus via the deposition of extracellular matrix [117, 118]. Developing glomerular endothelial cells branch extensively during this time and begin differentiating into fenestrated endothelia [1], as detailed in see vascular section below.

Renal Stroma

The renal stroma, like the nephrogenic mesenchyme, is derived from OSR1-positive intermediate mesoderm and the metanephric mesenchyme [119]. A hallmark of the initial renal stroma is expression of the transcription factor, *Foxd1*, which is seen as early as E11.5 in the mouse. The renal stroma is initially located at the periphery of the kidney and interdigitates between the developing nephron units and ureteric tips. One function of the early renal stroma is as a supportive framework for the developing vessels (described further below), nephron progenitors, and ureteric epithelia. As embryonic kidney development progresses, stromal cells are present in both the peripheral renal cortex and in the medulla surrounding developing collecting ducts. At this time, the cortical stroma expresses *Foxd1*, *Aldehyde dehydrogenase 1 family member A2 (Raldh2), β -Catenin, Retinoic acid receptor α* (*Rarα*), and *Rarβ2*, while the medullary stroma expresses *Fgf7*, *Pod1*, and *Bmp4* [119]. In addition, recent studies have shown heterogeneity of individual renal stromal cells using single cell RNA sequencing. Some of the stromally expressed genes and signaling pathways, such as β-catenin, are critical for stromal patterning. Other genes found in the stroma are critical for nephrogenesis and ureteric branching morphogenesis, as shown in mouse knockout studies. At birth, many of the developmental stromal cells have undergone apoptosis and are replaced by nephron segments such as loops of Henle. Many stromal derivatives do survive giving rise to fibroblasts, lymphocyte-like cells, glomerular mesangial cells, renin-expressing cells, vascular smooth muscle cells, pericytes, erythropoietin producing cells and a subpopulation of peritubular endothelial cells.

As noted, signaling from the renal stroma is critical for ureteric morphogenesis [119]. Three genes/pathways expressed within the stroma, retinoic acid, *Foxd1*, and *Pod1,* modulate ureteric branching by regulating expression of RET, a receptor tyrosine kinase expressed in ureteric tips and required for ureteric development (see below). Vitamin A is converted to its active form, retinoic acid, by the enzyme RALDH2 in the renal stroma. Moreover, blockade of retinoic acid signaling in mice, by deletion of *Raldh2* or by combined deletion of the retinoic acid receptors, *Rarα* and *Rarβ2*, leads to hypoplastic kidneys with a reduction in the number of ureteric branches; the ureteric branching defects are linked to downregulation of *Ret* expression in mutant embryos, which in the case of the retinoic acid receptor mutants can be rescued by forced re-expression of *Ret* in the ureteric tissues. *Foxd1* (expressed in cortical stroma and the renal capsule) or *Pod1* (found in medullary stroma) appear to appropriately restrict RET expression to ureteric tips; genetic deletion of either gene leads to mis-expression of RET throughout the entire ureteric tree and subsequent ureteric branching defects.

Non-autonomous actions of the stroma are also critical for nephron development. Mouse genetic studies show that *Foxd1* and *Pod1* are necessary for normal nephron patterning (in addition to ureteric morphogenesis) [89, 120]. Loss of *Foxd1* in mice leads to premature differentiation of stromal cells, which inhibits BMP7-mediated nephron progenitor differentiation [121]. Two studies showed that complete ablation of renal cortical stroma with diphtheria toxin led to abnormally thickened nephron progenitor caps and a decreased ability of progenitors to differentiate [122, 123]. Mechanistically, it appears that loss of the protocadherin FAT4 (when stroma is ablated) perturbs activity of the transcription factors, YAP and TAZ, which in turn disrupts WNT9b signaling and nephron differentiation [122]. Thus, in addition to providing a "framework" for the rest of the developing kidney, the renal stroma actively signals to other renal lineages and differentiates into cells that populate the mature kidney.

Two other derivatives of the renal stroma are cells that excrete renin and erythropoietin to regulate important biological processes throughout the body. The renin producing cells are found in the juxtaglomerular apparatus and regulate the renin-angiotensin system and have critical roles in mediating renal perfusion and GFR [124]. The erythropoietin producing cells in the kidney have been shown to arise from subsets of cortical stroma and their development and matruation are controlled by hypoxia induced factor signaling [125].

Vascular Development of the Kidney

Recent reviews have explored the origin and roles of the kidney vascular system [126, 127], which has become a hot topic as investigators attempt to vascularize renal organoids derived from induced pluripotent stem cells (see ▶ Chap. 4, "Stem Cells and Kidney Regeneration"). The adult kidney receives approximately 25% of the cardiac output. Furthermore, the adult kidney has a high complex vascular network with different functions and therefore different specialized endothelia depending on location [126]. Specifically, three major types of endothelial cells are present within the kidney, including fenestrated (in glomerular capillaries), fenestrated with diaphragms (in peritubular capillaries and ascending vasa recta) and continuous capillaries (in descending vasa recta) (Fig. 4). Not surprisingly, these various endothelial cell types have heterogeneous expression profiles and often appear to have different developmental origins. In addition to endothelial cells, the vasculature includes highly specialized mural cells adjacent to the endothelia; near the glomerulus. These mural cells include pericytes, renin cells, and fibroblasts, while those cells surrounding the peritubular capillaries consist of interstitial pericytes, fibroblasts and erythropoietin producing cells.

Angiogenesis Versus Vasculogenesis

Blood vessels can form by angiogenesis, in which new vessels sprout from existing vessels, or by vasculogenesis, in which *de novo* vessels form from endothelial progenitors. Extensive linage tracing experiments and transplantation studies have shown that both processes likely occur in renal vascular formation [126–130]. The early renal artery and efferent arterioles appear to be primary sites from which new angiogenic vessels sprout within the developing kidney. Simultaneously, renal endothelial progenitors (marked by *Flk1/Vegfr2*) form primitive vascular networks, particularly within the renal stroma, that subsequently join with and are pruned by the angiogenic vessels. The vasculogenic endothelial cell progenitors within the kidney appear to arise from the *Osr1*-expressing intermediate mesoderm, as is the case with the rest of the metanephric kidney. Finally, specification of the endothelium (whether angiogenic or vasculogenic in origin), including arterial, venous, capillary, or lymphatic fates is driven by growth factor signaling pathways and transcription factors, including VEGF, EPHRIN, NOTCH, and SOX [131].

Origins of the Peritubular Capillary Endothelia

While formation of glomerular capillaries has been extensively studied (see above), the origin of peritubular capillaries has been less well defined. Two studies, however, have found that peritubular capillaries arise from a combination of resident endothelial progenitors as well as invading angiogenic vessels [126, 127]. One intriguing study found that *Foxd1*-positive renal cortical stroma cells give rise to a subset of the peritubular endothelia but not the glomerular endothelia.

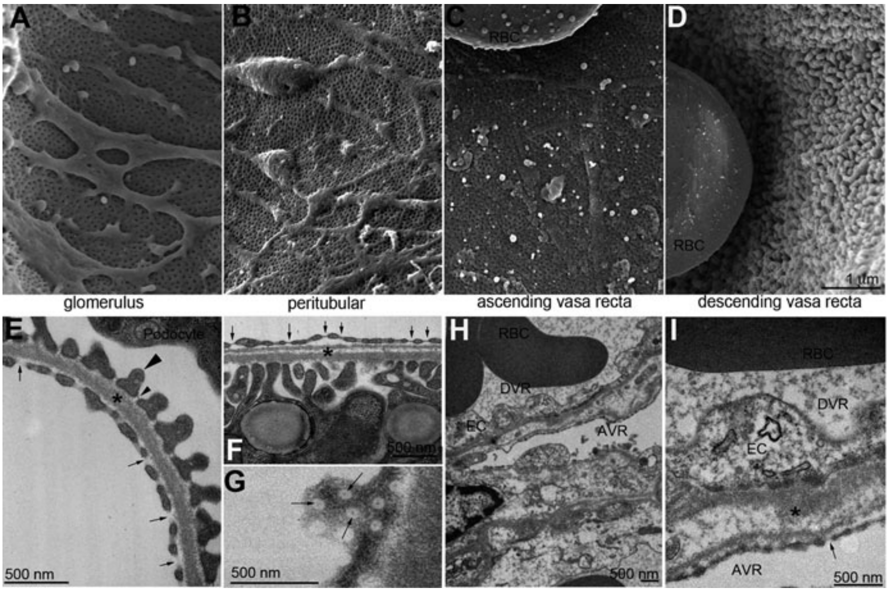

Fig. 4 **Electron microscopy demonstrating the varied renal endothelial cell types.** (**a, e**) Glomerular capillaries contain fenestrated endothelium without diaphragms (E, arrows) and share a basement membrane (*) with podocyte foot processes (large arrowhead) that are separated by slit diaphragms (small arrowhead). (**b, f, g**) Peritubular capillaries have fenestrated endothelial cells that are covered with diaphragms (**f**, **g**, arrows) and have a thick basement membrane (*) separating them from the tubular cells. (**c, h, i**) Ascending vasa recta (AVR) also have fenestrated endothelia with diaphragms (**c**, **h**, **i**, arrow). (**d, h, i**) Descending vasa recta (DVR) possess endothelia that are non fenestrated, but thick and continuous (**d**, **h**, **i**). *RBC* red blood cell, *EC* endothelial cell. Panels A–D= scanning electron micrographs. Panels E–I= transmission electron micrographs. (Reproduced with kind permission from Springer Science+Business Media. Stolz and Sims-Lucas [126], Figure 2)

These stromal cells have the potential to grow into endothelial cells within tumors when co-cultured with cancer cells and loss of these cells leads to kidney patterning defects in mice [132].

Molecular Control of Renal Vascular Development

A key signaling pathway mediating renal vascular development is the VEGF pathway. VEGF ligands are expressed early in the metanephric mesenchyme and later in the developing glomerular podocytes, distal tubules, and collecting ducts and at low levels in the proximal tubules [133, 134]. Developing endothelial cells, including those that arise from existing vessels and those forming *de novo*, express VEGF receptors; thus VEGF signaling appears to drive both angiogenesis and vasculogenesis within the kidney. Interestingly, *Vegfr2* is present on the apical surface of ureteric epithelium, which likely accounts for the stimulatory role of VEGF on ureteric growth (see below).

Hypoxia inducible factors (HIFs), a family of transcriptions factors, are likely master regulators of angiogenesis and vasculogenesis within the developing kidney [57, 135]. These molecules are activated during periods of low oxygenation, as occurs during embryogenesis, and are down regulated postnatally. The HIF genes are largely located in the nephrogenic zone, including podocytes, developing collecting ducts, and developing endothelial cells. HIF proteins induce expression of VEGF ligands, *Vegfr1* and *Vegfr2*, during kidney development by directly binding to hypoxia responsive elements on those genes.

Angiopoietin (ANG) growth factors that bind to TIE receptors also appear to have critical roles in renal vascular development and are at least in part regulated by HIF and VEGF signaling [136]. ANG1, which is expressed in the metanephric mesenchyme, maturing nephron tubules, and podocytes, signals through TIE2, which is expressed on endothelial cells. Conditional deletion of *Ang1* or *Tie2* in mice leads to glomerular capillary defects including endothelial cells that do not attach to the basement membrane. ANG2, expressed in vascular smooth muscle cells and pericytes, binds to TIE1 that is expressed by endothelial cells. Genetic deletion of *Ang2* leads to upregulation of TIE2 signaling and significant defects in renal peritubular capillaries.

Finally, the NOTCH signaling pathway appears to regulate renal angiogenic vessel outgrowth [137]. NOTCH receptors induce sprouting by stimulating expression of *Vegfr2* in vascular tip cells. Simultaneously, NOTCH inhibits VEGFR2 signaling in adjacent vascular stalk cells, causing them to remain dormant. Thus NOTCH regulates the pattern of branching in angiogenic vessels.

Collecting System Development

Ureteric bud formation begins in the fifth week of gestation in humans and at embryonic day 10.5 in mice. As noted previously, signals from the metanephric mesenchyme cause the ureteric bud to form from the nephric duct and then invade into the mesenchyme. Overall, collecting duct system development includes: (1) ureteric bud induction and outgrowth; (2) ureteric branching morphogenesis; and (3) cortical and medullary collecting duct patterning, all of which is discussed in more detail below.

Ureteric Bud Induction and Outgrowth

Failure of ureteric bud outgrowth results in renal aplasia, which can occur unilaterally or bilaterally. The *Glial cell line-derived neurotrophic factor* (GDNF) – *Ret proto-oncogene* (RET) signaling pathway is crucial for bud outgrowth. The receptor tyrosine kinase *Ret* and its co-receptor *Gdnf family receptor alpha 1* (*Gfrα1*) are expressed in the nephric duct, the initial ureteric bud and later in branching ureteric tips, while its ligand, *Gdnf*, is present in the metanephric mesenchyme (Fig. 6) [27]. Targeted deletion of *Gdnf, Ret,* or *Gfrα1* in mice generally results in bilateral renal aplasia due to a lack of ureteric bud outgrowth [27]. Heterozygous mutations of RET have also been identified in humans with bilateral renal aplasia, and a rare RET polymorphism has been reported in individuals with non-syndromic vesicoureteral reflux [138, 139]. The renal aplastic phenotype is not fully penetrant in a subset of *Gdnf*$^{-/-}$ or *Ret*$^{-/-}$ mutant mice [140, 141], suggesting that other molecular pathways also play a role in ureteric bud outgrowth. For example, genetic inactivation of several extracellular matrix (ECM) or ECM-interacting proteins such as members of the *Fras-Frem-Grip1* complex, *Laminin-5*, *Nephronectin* and *α8 integrin* also result in failure of ureteric bud outgrowth [27].

Many studies have focused on the molecular mechanisms that regulate GDNF-RET expression and/or signaling [27]. *In vitro* studies with GDNF-soaked agarose beads show that the entire length of the nephric duct is competent to respond to GDNF by initiating ectopic ureteric bud formation [142, 143]. Moreover, mice that ectopically express *Gdnf* or *Ret in vivo* develop renal malformations such as duplex kidneys and hydronephrosis [144, 145]. Together, these data show that GDNF-RET signaling must be tightly spatially regulated for the ureteric bud to form in the correct location from the nephric duct, ensuring that paired single kidneys form at the appropriate position in the body (Fig. 5).

Several genes are thought to be crucial in restricting *Gdnf* to the posterior intermediate mesoderm including, *Forkhead box c1* (*Foxc1*), *Foxc2*, *Slit guidance ligand 2* (*Slit2*), and *Roundabout Guidance Receptor 2* (*Robo2*) (Fig. 5) [27]. Homozygous mutant mice for these genes develop ectopic ureteric buds, multiple ureters and hydroureter, as a result of ectopic *Gdnf* expression. *Foxc1* and *Foxc2* encode transcription factors that suppress *Gdnf* expression in the metanephric mesenchyme. In the developing kidney, *Slit2* is expressed in the nephric duct, and *Robo2* is detected in the metanephric mesenchyme. Together, the SLIT2-ROBO2 pathway is thought to regulate the bioavailability of *Gdnf*. ROBO2 mis-sense mutations in humans have been identified in families with vesicoureteral reflux and/or duplex kidneys [146].

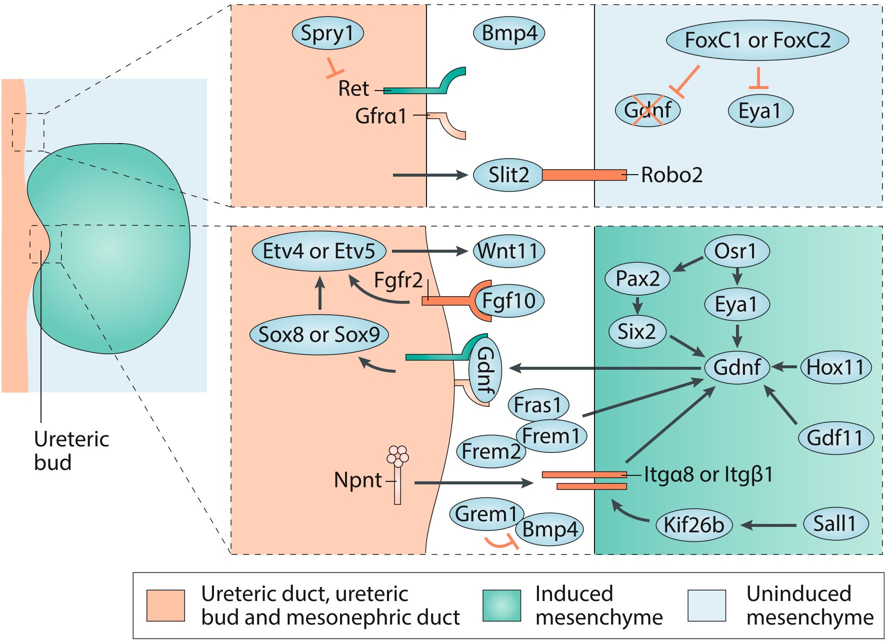
Spry1
Bmp4
FoxC1 or FoxC2
Ret
Gfrα1
Gdnf
Eya1
Slit2
Robo2
Etv4 or Etv5
Wnt11
Fgfr2
Fgf10
Osr1
Pax2
Eya1
Sox8 or Sox9
Six2
Gdnf
Gdnf
Hox11
Fras1
Frem1
Frem2
Gdf11
Npnt
Itgα8 or Itgβ1
Grem1
Bmp4
Kif26b
Sall1
Ureteric bud
Ureteric duct, ureteric bud and mesonephric duct
Induced mesenchyme
Uninduced mesenchyme

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Fig. 5 Molecular determinants of ureteric bud formation. Ureteric bud outgrowth is dictated by a complex genetic network centered around interactions between Gdnf and its receptors Ret and Gfrα1. This schematic shows some of the principal players in this process, many of which act to suppress inappropriate Gdnf production and/or signaling downstream of Ret along the nephric duct, except at the site of ureteric bud induction. Loss of these controls often results in ectopic ureteric bud formation. Ureteric bud outgrowth at the level of the embryonic hindlimbs is facilitated at around embryonic day (E) 10.5 (E24 in humans) by the concerted actions of several growth and transcription factors that result in the production of Gdnf in the metanephric mesenchyme and the reciprocal activation of Ret signaling. (Short and Smyth [27], adapted from Figure 3)

Two other genes, *Sprouty1* (*Spry1*) and *Bone morphogenetic protein 4* (*Bmp4*), modulate the response of ureteric bud epithelial cells to GDNF-RET signaling (Fig. 5) [147]. Loss of *Spry1*, which is normally expressed in the nephric duct, results in ectopic ureteric bud induction, multiple ureters, multiplex kidneys, and hydroureter [147, 148]. *Spry1* mutant embryonic kidneys have increased expression of *Gdnf* and GDNF-RET target genes, and have increased sensitivity to GDNF-induced ureteric induction in organ culture. Mice heterozygous for *Bmp4* have ectopic or duplicated ureteric buds, resulting in hypo-dysplastic kidneys, hydroureteronephrosis and ureteral duplications. BMP4 mutations have also been described in humans with renal tract malformations [149]. *Bmp4* expression itself is inhibited by *Gremlin 1* (GREM1), and together with aforementioned genes, functions to ensure that only one ureteric bud is induced from the correction position along the nephric duct [147].

The downstream effects of GDNF-RET signaling, namely, ureteric bud proliferation, survival, and ureteric outgrowth and branching are mediated by several transcription factors, including *SRY box transcription factor 8* (*Sox8*), *Sox9*, *Ets variant transcription factor 4* (*Etv4*) and *Etv5*. Combined deletion of *Etv4* and *Etv5* causes bilateral renal aplasia in mice [148]. *Etv4* and *Etv5* drive expression of several critical genes in the ureteric bud tip, including *Wnt family member 11* (*Wnt11*), *C-X-C motif chemokine receptor 4* (*Cxcr4*), *Matrix metallopeptidase 14* (*Mmp14*), *Myb proto-oncogene* (*Myb*) and *Met proto-oncogene Met* [148]. Furthermore, genetic deletion studies in mice have shown that *Wnt11* is necessary for normal *Gdnf* expression in the metanephric mesenchyme [150].

Ureteric Branching Morphogenesis

After growing into the metanephric mesenchyme, the ureteric bud bifurcates into a T-shaped structure. The ureteric bud then continues to branch, ultimately generating about 15 generations of branches, with the earliest branches remodeling to form the calices and renal pelvis [27]. The process of ureteric branching includes: (1) expansion of the ureteric bud at its leading tip (termed the ampulla); (2) division of the ampulla to form new branches; and (3) elongation of newly formed branches (also referred to as the ureteric trunk). In humans, during the first 9 generations of branching, ureteric bud tips induce formation of new nephrons from the surrounding cap mesenchyme at about a 1:1 ratio [1]. Ureteric bud branching is completed by the 20th—22nd week of human gestation, and subsequent collecting duct maturation occurs by elongation of peripheral (cortical) segments and remodeling of central (medullary) segments. At this stage, four to seven new nephrons are induced around each tip of a terminal collecting duct branch [1, 5].

Localized cell proliferation occurs in both the ureteric tip and ureteric trunk compartments; however, time lapse imaging has demonstrated that a subpopulation of ureteric tip progenitors remain in the tip niche to undergo sustained proliferation and drive subsequent branching [27]. Interestingly, ureteric tip cells undergo intraluminal mitosis, an unusual feature for epithelial proliferation. Cell survival is also critical for normal renal branching morphogenesis; defects in cell survival are associated with renal cystic dysplasia and urinary tract dilatation. Moreover, targeted deletion of the apoptosis regulator *Bcl2* [151] and *Transcription factor Ap-2* (*AP-2*) [152], genes critical for cell survival, results in increased apoptosis and collecting duct cysts in mice.

Several signaling pathways are necessary for branching morphogenesis. In addition to its role in ureteric bud induction, GDNF-RET signaling is critical for ureteric branching and appears to mediate dynamic changes in cell migration at the ureteric tip [27, 147]. In chimeric embryos with cells of varying *Ret* activity, the cells that express the highest level of *Ret* move to the ureteric bud tip, closest to where *Gdnf* is expressed by the metanephric mesenchyme. *Wnt11* (expressed in ureteric tips) is necessary for maintaining normal *Gdnf* expression; conversely, *Wnt11* expression is reduced when GDNF signaling is absent. Furthermore*,* *Wnt11* mutant mice have defective ureteric branching morphogenesis, and thus develop renal hypoplasia. Finally, conditional targeting of *β-catenin*, a key mediator of canonical WNT signaling, in the ureteric lineage results in aberrant branching, loss of ureteric bud tip gene expression, and premature expression of differentiated collecting duct genes.

Fibroblast growth factor (FGF) signaling is also critical for ureteric branching. *In vitro* studies have shown that exogenous FGF ligands differentially modulate ureteric bud growth and proliferation [153]. FGF10 preferentially stimulates proliferation at ureteric bud tips, whereas FGF7 increases cell proliferation throughout the developing collecting ducts. *In vivo*, global deletion of *Fgf7* or *Fgf10* in mice results in ureteric branching defects and hypoplastic kidneys. Conditional targeting studies in mice have revealed that fibroblast growth factor receptor 2 (FGFR2) is likely the key FGF receptor mediating effects on ureteric branching [154]. Loss of *Fgfr2* in the ureteric bud results in hypoplastic ureteric ampullae with reduced proliferation and increased apoptosis, ultimately leading to a significant reduction in ureteric branching and hypoplastic kidneys. In addition, another study revealed that while combined loss of *Sprouty1* and *Gdnf* in mice largely rescues ureteric defects compared to when either locus is deleted alone, additional loss of *Fgf10* in the combined mutants led to complete loss of ureteric branching and renal aplasia [148]; thus, FGF signals appear to be able to largely substitute for GDNF in promoting ureteric morphogenesis in the absence of *Sprouty1*. Finally, a combination of *in vitro* and *in vivo* experiments revealed that FGF signaling acts in a coordinated fashion with *Wnt11* and *Gdnf* to regulate ureteric morphogenesis, in concert with *Sprouty* genes [155].

Advances in imaging techniques that permit detailed analysis of the morphometric parameters of intact developing kidneys have allowed researchers to quantify branching morphogenesis *in vivo* [27]. These approaches have provided insights into how well the branching pattern of the developing kidney is conserved, with relative uniformity and increases in branching complexity observed early in kidney development. Recently, factors that regulate the spatial arrangement of different branches in the kidney have also been described. Moreover, branching in the kidney often occurs through asymmetric branching that arise through suppression of bifurcations between growing ureteric tips and that is not impacted by nephron formation [19, 156]. The reaction-diffusion model described by Turing in 1952 proposed a theoretical mechanism to explain how spatial patterns could form from the interaction of two diffusible substances, even from an initial random or uniform condition [157]. Mathematical modeling of embryonic kidney explants has shown that ureteric branching is most compatible with a ligand-receptor based Turing-type model based on GDNF-RET signaling; furthermore, WNT11-driven maintenance of GDNF expression appears important in determining the distance between forming ureteric buds [158].

Cortical and Medullary Collecting Duct Patterning

From the 22nd-34th week of human gestation [1] and embryonic day 15-birth in mice [5], the cortical (peripheral) and medullary (central) regions of the kidney become established. The relatively compact, circumferential renal cortex comprises approximately 70% of the mature kidney volume [159]. The renal medulla develops as a modified cone shape, and occupies the remainder of the mature kidney volume. The apex of the medullary cone consists of collecting ducts converging in the inner medulla, and is termed the papilla. Ultimately, medullary collecting ducts become morphologically distinct from cortical collecting ducts. Medullary collecting ducts become elongated, linear, and remain relatively unbranched in a region devoid of glomeruli. In contrast, collecting ducts in the renal cortex remain branched and during development, induce nephrogenic cap mesenchyme to form nephrons throughout nephrogenesis.

These morphological differences are likely due in part to distinct axes of growth in the developing renal cortex and medulla. The renal cortex grows circumferentially, which preserves the organization of the peripheral tissues, including differentiating glomeruli, nephron tubules, and collecting ducts [159]. In contrast, the developing renal medulla expands longitudinally, perpendicular to the axis of cortical growth, due to elongation of outer medullary collecting ducts. Stromal cells may be a source of stimulatory cues for the medullary growth [159]; studies have shown that mice lacking the stromal transcription factors *Forkhead box D1* (*Foxd1*) and *Transcription factor 21* (*Tcf21*) have abnormal medullary collecting duct patterning [160]. Finally, apoptosis appears to participate in physiological remodeling the branched medullary ureteric tissues into elongated tubules, as programmed cell death normally occurs prominently in the developing medullary ureteric epithelia that become the papilla, calyces, and renal pelvis [51].

Multiple genes have been implicated in the differentiation of cortical and medullary collecting ducts, including those that encode for soluble growth factors (*Fgf7*, *Fgf10*, *Bmp4*, *Bmp5* and *Wnt7b*), proteoglycans (*Glypican 3* (*Gpc3*)), cell cycle regulatory proteins ($p57^{KIP2}$), and components of the renin-angiotensin axis (*Angiotensin* [*Agt*] and *Angiotensin type 1* and *2 receptors* [*Agtr1* and *Agtr2*]). *Fgf7* mutant mice have marked papillary underdevelopment, while *Fgf10* null kidneys exhibit medullary dysplasia with fewer loops of Henle and medullary collecting ducts, increased medullary stroma, and enlargement of the renal calyx [153]. The ability of FGF ligands to bind properly to their receptors require interactions with cell surface proteoglycans, including glypicans. GPC3 is required for normal medullary patterning in humans and mice [153]. Moreover, the medullary dysplasia observed in *Gpc3* deficient mice appears to result from unrestrained proliferation and overgrowth of the ureteric bud and collecting ducts, followed by aberrant apoptosis [161, 162]. The *Gpc3-/-* mouse medullary defects appear to be driven by altered responses of mutant collecting duct cells to growth factors including FGFs [162]. Finally, mice lacking the cell cycle protein $p57^{KIP2}$ demonstrate medullary dysplasia, with fewer inner medullary collecting ducts [163]. Together, these studies reveal the importance of balanced cell proliferation and apoptosis in medullary collecting duct patterning.

Proper elongation and growth of medullary collecting ducts also appears to rely on oriented cell divisions. Studies have shown that canonical WNT signaling in collecting ducts via WNT7b is required for the formation of the renal medulla, and leads to properly oriented cell division and survival [147, 164]. Furthermore, α3β1 integrin and the receptor tyrosine kinase c-MET act in concert to regulate *Wnt7b* expression and signaling in medullary collecting ducts [147].

Finally, angiotensin and angiotensin receptors appear critical for development of the renal calyces, pelvis, and ureter. Mice lacking *Agt* or *Agtr1* genes demonstrate progressive widening of the calyx, and atrophy of the papillae and underlying medulla [160]. These defects appear to be caused by decreased proliferation of the smooth muscle cells that line the renal pelvis. Loss of *Agtr2* causes a range of renal anomalies secondary to ureteric mispatterning, including vesicoureteral reflux, duplex kidneys, renal ectopia, uretero-pelvic or uretero-vesical junction stenoses, renal dysplasia or hypoplasia, multicystic dysplastic kidneys, and renal aplasia [160]. The renin-angiotensin system also appears to regulate ureteric bud branching morphogenesis, papillary collecting duct proliferation and survival, and the expression of multiple genes implicated in renal medulla development [147].

Lower Urinary Tract Development

Anatomic and Functional Development

Concurrent with metanephric kidney development, the embryonic ureter and bladder form, the former functioning to propel urine into the latter, which stores urine until an appropriate time to expel it via the urethra. Similar to the kidney, the ureter and bladder undergo maturation largely due to reciprocal interactions between an epithelium (i.e., urothelium) and surrounding mesenchyme that forms the lamina propria, muscle, and adventitia (Fig. 6). For reviews on anatomic and molecular control of lower urinary tract development, see [165–167].

Ureter development begins simultaneously with metanephric kidney development around the fifth week of gestation in humans and at E10.5 in the mouse, when the ureteric bud arises from the nephric duct [165, 168]. Thus the embryonic origin of the ureteral urothelium is the intermediate mesoderm, the same as the metanephric kidney. By E11.5, the ureteric bud has been segmented into a distal portion that will develop into the ureter and a proximal end that has invaded the metanephric mesenchyme to eventually branch and form the collecting ducts and renal pelvis (see above). The mesenchyme surrounding the early developing ureter consists largely of tailbud-derived mesenchyme that appears to be crucial for directing the distal ureteric bud toward a ureter fate [165]. Between E10.5 and 13.5, the nascent ureter transitions from attaching to the nephric duct to emptying directly into the early bladder (see below). By E13.5, a thin outer ring of ureteral mesenchyme condenses and expresses alpha smooth muscle actin (αSMA) mRNA, the first marker of differentiation toward a smooth muscle fate [169]. Expression of αSMA protein is not noted until E14.5 in the proximal portion of the ureter (nearest the kidney) and then throughout the entire length of ureter by E16.5; thus, ureter muscle development progresses in a rostral to caudal direction [165]. Concurrent with mesenchymal development, the ureteral urothelium gradually matures from a simple epithelium to a stratified epithelium consisting of at least three cell types with distinct structural features and molecular markers: Basal, Intermediate, and Superficial/Umbrella cells (Fig. 7) [166]. The cell layer that houses embryonic ureter urothelial progenitors is not entirely clear. One mouse study suggests that urothelial Basal cells, arising from the original ureteric epithelium, serve as the progenitors for other ureteral urothelial cell types [165]. A more recent mouse lineage tracing study suggests that both Superficial and Basal cells arise from an Intermediate cell progenitor [166]. A unique feature of mature urothelium (compared with other epithelia) is that Superficial cell and subsets of Intermediate cells express urothelial plaques, consisting of uroplakin (UPK)

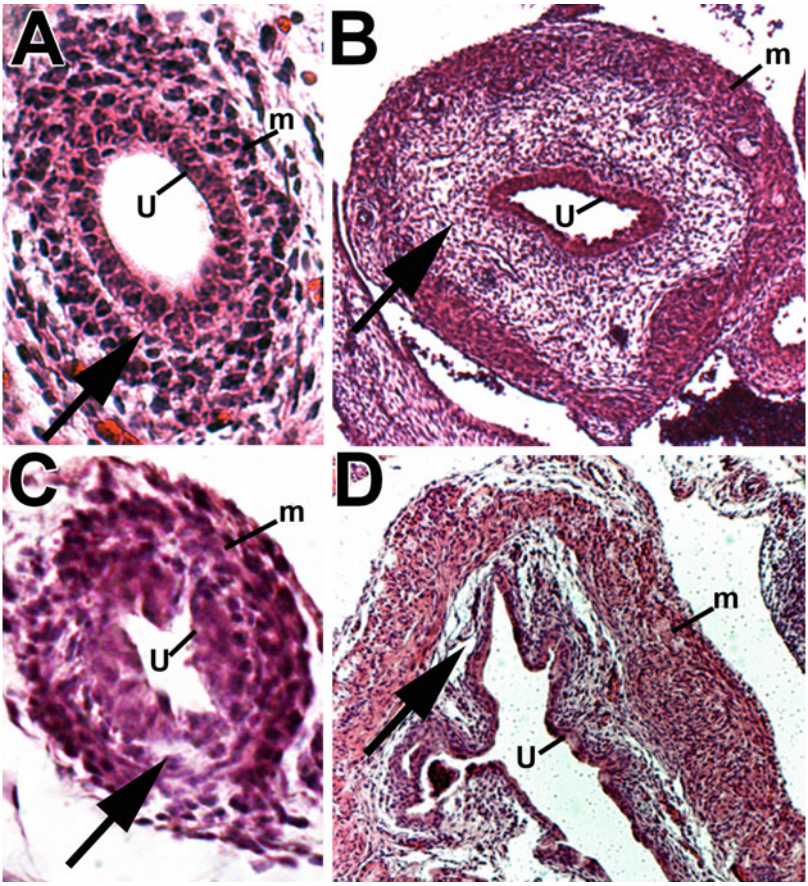

Fig. 6 H&E stained sections from E15.5 and P1 mouse ureters and bladders. (**a**, **b**) E15.5 ureters (**a**) and bladders (**b**) have early urothelium (u), an inner layer of mesenchyme (future lamina propria, arrows), and an outer layer of condensing mesenchyme (future muscle, m). (**c**, **d**) P1 ureters (**c**) and bladders (**d**) have a more stratified urothelium (u) and a well-developed lamina propria (arrows) and outer muscle (m). The adventitial layer of fibroblasts that surrounds the muscle in both tissues is not labeled. **a**, **c** × 100 magnification; **b**, **d** × 40 magnification

proteins (Fig. 7), which provide a permeability barrier and act as a binding site for uropathogenic E. coli (among other functions) [166].

An important function of the ureter is to continuously propel urine from the renal pelvis to the bladder. The ability of the ureter to undergo peristaltic waves of contraction followed by relaxation appears to be intrinsic and not dependent on urine flow; cultured explants of E13.5 mouse ureters attached to kidneys begin to undergo spontaneous peristaltic contractions within a few days, as do isolated and cultured E15.5 ureters [165]. Elegant studies identified a population of modified smooth muscle cells that act as pacemakers at the junction of the renal pelvis and the kidney and that express hyperpolarization-activated cation–3 (HCN3) channels (a family of channels that are also present in cardiac pacemakers) [165]. Loss of HCN3 activity in mice leads to abnormal coordination and frequency of ureter contractions [165]. Following this study, another described a population of secondary pacemakers that are located in the muscle of the mouse proximal ureter (starting at the ureteropelvic junction), and that have morphological and molecular features similar to intestinal pacemakers including expression of *c-Kit* [165]. As with *Hcn3*, knockdown of *c-Kit* expression led to cessation of ureteral peristalsis [165]. Thus, like the cardiac conduction system, the ureter has primary and secondary pacemakers that act to drive urinary propulsion in a coordinated fashion.

The embryonic bladder initially forms around the fifth gestational week in humans and at E11.5–12.5 in the mouse [167, 168]. Unlike the ureter, bladder urothelium is derived from endodermal cloacal tissues. Starting at E10.5, the cloaca becomes divided by the urorectal septum into a dorsal anorectal canal and a ventral urogenital sinus. At E11.5–12.5, the urogenital sinus further subdivides into an anterior

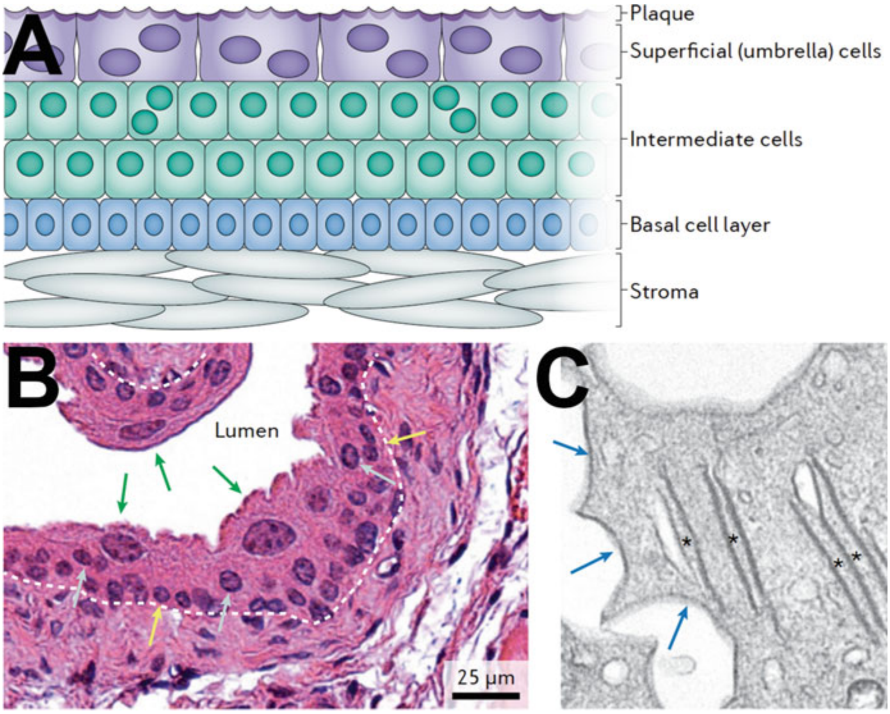

Fig. 7 **Structure of adult urothelium.** (**a**) Diagram showing the various cell layers of the mature urothelium, including Superficial cells that possess an apical plaque, Intermediate cells and Basal cells that sit on the stroma. (**b**) Hematoxylin and eosin stained image of urothelium demonstrating the large Superficial cells with polyploid nuclei (green arrows), Intermediate cells (gray arrows), and Basal cells (yellow arrows). Dashed white line indicates the border of the urothelium and underlying stroma. ×40 magnification (**c**) Transmission electron microscopy of Superficial cells shows their fusiform uroplakin-containing vesicles (asterisks) and concave apical plaques (blue arrows). ×3000 magnification. (Reprinted by permission from Springer Nature. Jackson et al. [166], adapted from Figure 1)

portion, which will become the bladder, and a posterior portion that forms the urethra and parts of the female vagina. The mesenchyme that surrounds bladder urothelium is largely thought to be from splanchnic mesoderm, although fate-mapping studies reveal that tailbud mesenchyme also contributes to bladder mesenchyme (similar to the ureter) [165]. By E13.5, the bladder is recognized as a distinct structure that is attached directly to the ureters. As is the case with ureters, αSMA mRNA expression in the developing bladder muscle precedes protein expression; mRNA expression appears as early as E11.5 in mice [169], while protein expression begins at E13.5 [170]. Unlike the ureter (and many other organs with smooth muscle such as the intestine) that has a thin ring of mesenchyme that begins to differentiate into muscle, the entire outer half of the bladder mesenchyme condenses simultaneously and strongly expresses αSMA by E15.5 [169]. Similar to the ureter, bladder urothelium matures from a simple epithelium to a stratified epithelium, consisting of cell types similar to the ureter, including having Superficial cells and subsets of Intermediate cells that express uroplakins and urothelial plaques [166]. While bladder urothelial Basal cells were originally thought to be the progenitor cell for the other urothelial cell types, lineage tracing studies revealed the presence of a transient population of "P" cells that appear to serve as embryonic progenitors for uroplakin-expressing Intermediate and Superficial cells [166]. In contrast Keratin 5-expressing Basal and/or Intermediate cells appear to give rise to themselves and not uroplakin-expressing cells during development [166].

While the connection of the ureters with the bladder is critical, how this occurs has been the subject of some debate. What is clear is that between E12.5 and 13.5 in the mouse, the common nephric duct (caudal portion of the nephric duct between the ureter base and future bladder) moves adjacent to the bladder, allowing for the ureters and rostral nephric ducts (future male gonadal excretory ducts) to separate and empty directly into the bladder. The triangular portion of the bladder demarcated by the entry points of the ureters and the bladder neck (where the remaining embryonic nephric ducts empty) is known as the trigone. Historically the common nephric duct was thought to become incorporated into the bladder to form the trigone, given that the mature trigonal epithelium is smooth while the rest of the bladder urothelium has rugae [167]. However, fate mapping studies in mice revealed that the common nephric duct undergoes complete apoptosis starting at E12.5, and that the urothelium of the trigone originates entirely from bladder urothelium [167]. The conclusions from the fate mapping studies were supported by tissue studies comparing how mouse embryonic trigonal epithelium, nephric duct epithelium and bladder dome epithelia differentiated when combined with rat urogenital sinus mesenchyme and grown under kidney capsule; moreover, at the conclusion of the experiments, the trigonal epithelium expressed differentiation markers similar to bladder dome epithelium and not the nephric duct epithelia [167]. Regarding the origin of the mesenchymal cells underlying the bladder trigone, lineage tracing studies using Cre-/loxP techniques in mice showed that the muscle present in the trigone arises mostly from the bladder with only a few fibers emanating from ureteral muscle [167]. Thus, the trigone originates almost completely from bladder tissues and not the nephric duct.

Recently, a group of investigators identified the presence of modified urothelial cells that line the inner renal medulla (termed “intrarenal urothelium”) [166]. In the quiescent state, the intrarenal urothelium consists of one to layers of interspersed Uroplakin 3^+ and Keratin 5^+ cells and is devoid of urothelial plaques. With urinary tract obstruction, however, the intrarenal urothelium can proliferate and stratify, including generating large Superficial-like cells that have organized uroplakin-derived plaques. After genetic deletion of uroplakins in the intrarenal urothelium, mice subjected to urinary tract obstruction develop severe hydronephrosis and biochemical evidence of renal failure beyond what is seen in controls; this suggests a critical roles for the intrarenal urothelium with high urinary pressures. Fate mapping studies have shown that Keratin 5^+ cells serve as the embryonic progenitors for the uroplakin-expressing cells within the intrarenal urothelium. After postnatal day 14 in mice, Keratin 5^+ cells lose the potential to form uroplakin-expressing cells, except after urinary tract obstruction when the former are again able to give rise to the latter. Presently, signaling pathways required for morphogenesis of the intrarenal urothelium are unknown. However, many studies have identified molecules required for ureter and bladder development as will be discussed next.

Molecular Control of Ureter and Bladder Development

Ureter

The pathways critical for early ureteric bud formation were already covered in the section on kidney development above. Once the ureteric bud forms, the distal end matures into collecting duct epithelium while the proximal end (near the bladder) becomes ureter urothelium. Studies have shown that bone morphogenetic protein 4 (BMP4), which is secreted by tailbud derived mesenchyme surrounding the distal ureteric bud, drives the epithelium toward a urothelial fate; moreover, ectopic *Bmp4* expression around proximal portions of the ureteric bud directs it to become urothelium instead of collecting duct epithelium [165]. More recent work has shown that in addition to specification, BMP4 is required for ureter urothelial proliferation and differentiation, mostly via AKT signaling (with minor contributions from P38 kinase and ERK signaling) [171]. Another study identified the signaling pathway that drives BMP4 expression [172]. The growth factor Sonic hedgehog (SHH) is secreted by the ureteral epithelium and binds to its receptor, Patched 1, in surrounding mesenchyme. This in turn drives expression of the Forkhead transcription factor gene, *Foxf1*, that then stimulates BMP4 expression. Mutations in *SHH*, *FOXF1* and *BMP4* have been identified in some patients with renal and lower urinary tract anomalies, attesting to the clinical relevance of this signaling axis [166]. In addition to the SHH-FOXF1-BMP4 signaling axis, uroplakins, markers of urothelial maturation, are also critical for normal urothelial morphogenesis. Loss of either Uroplakin II or Uroplakin IIIa leads to severe urothelial plaque defects, hypoplastic Superficial cells, urothelial leakiness, hydronephrosis, and vesicoureteral reflux [165]. Moreover, mutations in *UPKIIIa* in humans have been associated with severe renal dysplasia and reflux [173], whereas the role of *UPKII* in humans is still unclear [174].

Many of the molecules critical for ureter mesenchymal patterning have also been identified. In addition to its role in ureter urothelial morphogenesis, Sonic hedgehog secretion by the urothelium is also critical for ureter mesenchyme development. Conditional ablation of *Shh* in developing mouse ureteric epithelium leads to loss of mesenchymal proliferation and smooth muscle differentiation [165]. Furthermore, SHH signaling from urothelium has also been shown to be critical for formation of *Hcn3* and *c-Kit* expressing pacemakers within the ureteral muscle, via the hedgehog signaling mediators Smoothened and GLI3 repressor [165]. In addition, the aforementioned SHH-FOXF1-BMP4 signaling axis is necessary for ureter mesenchyme proliferation and differentiation into muscle, especially at the ureterovesical junction [172]. The downstream signaling target of BMP4 most critical for ureter mesenchymal proliferation is AKT while the target most important for muscle differentiation is SMAD 1/5/9 (P38 kinase has minor roles in both) [171]. Finally, the transcription factor, Teashirt 3 (*Tshz3*), downstream of both SHH and BMP4, is critical for proximal ureteral smooth muscle differentiation and for normal ureteral peristaltic function; deletion of *Tshz3* in mice leads to severe muscle patterning defects and congenital hydronephrosis [165]. Thus, SHH signaling is critical for ureter mesenchyme morphogenesis.

Another signaling pathway that is critical for ureter mesenchyme patterning is the WNT- T-box (TBX) transcription factor signaling axis. WNT ligands are secreted by urothelium, bind to frizzled receptors in the mesenchyme and signal primarily by stabilization of β-catenin (CTNNB1). Investigators found that genetic ablation of *Ctnnb1* in mice led to decreased mesenchymal proliferation and differentiation into smooth muscle cells, with concurrent expansion of the outer adventitial fibroblast layer [165]. More recently other groups found that the transcription factors, TBX2 and TBX3 are critical WNT targets in ureter mesenchyme; the TBX proteins in turn suppress the WNT antagonists, DKK2 (Dickkopf 2), and SHISA2, and stabilize BMP4 expression by suppressing the BMP4 antagonist, BMPER (BMP binding endothelial regulator), together leading to proper ureter mesenchymal patterning and differentiation [175]. Another T-box transcription factor, TBX18 is also induced by WNT signaling and is required for ureter mesenchyme morphogenesis. Genetic ablation of *Tbx18* leads decreased levels of *Sox9* and to decreased proliferation and failure of smooth muscle differentiation, and ultimately severe hydroureteronephrosis [165]. Recently, investigators identified dominant negative mutations in *TBX18* in three families leading to renal and urinary tract anomalies including severe ureteropelvic junction obstruction (validating the role of TBX18 in human disease) [176].

Other signaling molecules have been shown to regulate ureter mesenchyme development. Studies in mice found that conditional ablation of the calcineurin b1 subunit in developing ureteral mesenchyme led to reduced smooth muscle proliferation and hydronephrosis [165]. Furthermore, genetic ablation of the angiotensin type 1 receptor (which is expressed in ureteral mesenchyme) caused smooth muscle hypoplasia, a lack of peristalsis, and ultimately hydronephrosis [165]. Retinoic acid (RA) receptor signaling has been shown to be critical for maintaining ureter mesenchymal progenitors, as reduced retinoic acid signaling leads to premature ureter smooth muscle differentiation [177]. Conversely, *Gata2*, a transcription factor in ureter mesenchyme that is induced by retinoic acid, appears to drive feedback inhibition of RA signaling; conditional deletion of *Gata2* in ureter mesenchyme led to a delay in ureter smooth muscle differentiation and hydroureter apparently from disinhibited retinoic acid receptor signaling [177]. Lastly, Discs-large homolog 1 (DLG1) is the only gene identified to date that is necessary for ureter lamina propria/stromal cell development; genetic deletion of *Dlg1* in mice led to an absence of the entire ureteral lamina propria layer and disorganized muscle development [178].

Bladder

Compared with the ureter, less is known about the molecular control of bladder development. Some of the pathways critical for formation of the urogenital sinus (future bladder) have been identified. Bidirectional signaling between Ephrin-B2 (ligand) and EPHB2/EPHB3 (tyrosine kinase receptors) is critical for septation of the cloaca into the ventral urogenital sinus and the dorsal anorectal canal [179]. Sonic hedgehog signaling is also critical for formation of the urogenital sinus. In mice, the loss of *Shh* or compound mutations in the downstream hedgehog mediators, *Gli2* and *Gli3,* lead to failure of cloacal septation [180]. During later stages of bladder formation, urothelial cell stratification is dependent on retinoid signaling emanating from the lamina propria surrounding the urothelium; forced expression of a dominant negative retinoic acid receptor in developing mouse urothelium leads to a loss of Superficial cells and subsets of Intermediate cells [166]. Finally, a recent study revealed that the EED (Embryonic Ectoderm Development) subunit of the polycomb repressive complex 2 (PCR2), which leads to transcriptional silencing of genes, is required for normal bladder urothelial morphogenesis [181]. Conditional deletion *Eed* in embryonic mouse urothelium led to reduced urothelial proliferation and differentiation defects, apparently due in large part to reduced *Shh* expression [181].

In addition to data on signaling necessary for bladder urothelial development, some of the genetic pathways critical for bladder mesenchyme development have been identified. As is true in the ureter, several studies have shown that SHH signaling from the bladder urothelium is necessary for bladder mesenchyme morphogenesis; loss of *Shh* in mice leads to a complete loss of bladder smooth muscle formation [167]. Moreover, the gradient of SHH signaling is critical for normal mesenchymal patterning; smooth muscle formation is inhibited in favor of collagen in the bladder mesenchyme nearest to the urothelium (where SHH signal is the strongest). In contrast, smooth muscle formation is stimulated in outer regions of bladder mesenchyme, where SHH signal is weaker [167]. Elegant studies have shown that co-culture of embryonic mouse bladders with fetal esophagus (which secretes high levels of SHH) leads to repression of muscle formation in the outer bladder mesenchyme that normally forms the detrusor muscle [167]. The differential effects of SHH signal intensity of the fate of bladder mesenchyme appears to be secondary to BMP4 signaling; SHH-Patched signaling induces BMP4 expression in the mesenchyme nearest the urothelium, which suppresses muscle formation, while bladder mesenchyme farthest from the urothelium is relatively devoid of SHH-induced BMP4 expression. Another study showed that fibroblast growth factor receptor 2 (FGFR2) expressed in bladder mesenchyme alters the sensitivity of the mesenchyme to SHH signals. Conditional loss of *Fgfr2* in mouse embryonic bladder mesenchyme led to an expansion of the collagen-rich lamina propria and loss of outer detrusor muscle [182]; loss of FGFR2 led to enhanced expression of *Boc* and *Cdo*, genes that encode for co-receptors that enhance SHH signaling through its Patched receptors. The postnatal mutant mice had poorly compliant bladders and severe voiding dysfunction.

Other molecules critical for bladder mesenchyme patterning have been identified. Transforming growth factor beta (TGF-β), which is in the same superfamily of signaling molecules as BMPs, appears critical for bladder muscle patterning. Chemical inhibition of TGF-β1 signaling suppressed bladder muscle formation in embryonic mice [167]. Another group has shown the importance of myocardin, a transcription factor, in bladder smooth muscle patterning [169]. The investigators generated random transgene insertional mutant mice called megabladder mice (*mgb-/-*) that have very limited αSMA expression in embryonic bladders, and ultimately develop massive bladders with no functional detrusor.

The group later found that the mouse has significantly reduced levels of myocardin. Importantly, the investigators recently identified four families with members afflicted with bladder smooth muscle defects leading to megabladders that have mutations in the myocardin gene, revealing the clinical relevance of the molecule [169].

Ureter-Bladder Anastomosis

There are some data on genetic pathways necessary for the proper connection between the ureter and bladder, i.e., apoptosis of the common nephric duct starting at E12.5 in mice. Retinoid signaling from the bladder has been shown to be critical for this process. Genetic ablation of retinaldehyde dehydrogenase-2, an enzyme expressed in the urogenital sinus and necessary for retinoic acid synthesis, led to persistence of the common nephric duct with ureters ending blindly in the nephric duct [167]. In addition, the receptor tyrosine kinase, RET, has also been shown to be essential for ureter-bladder anastomosis. Mice with a point mutation in tyrosine 1015, the phospholipase Cγ binding site on RET (*RetY1015*), have a persistent common nephric duct due to enhanced proliferation and decreased apoptosis [183].

Summary

Lower urinary tract defects contribute substantially to chronic kidney disease in children, necessitating a better understanding about the genes regulating lower urinary tract morphogenesis. While our understanding of the molecular control of ureter, bladder, and intrarenal urothelial development trails that of the kidney, more studies are emerging on how these tissues form.

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Postnatal Renal Maturation

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Michel Baum and Melissa Anslow

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Abstract

The kidney functions to maintain a constant volume and composition of the extracellular fluid volume. The glomerulus forms an ultrafiltrate of plasma which enters the proximal tubule where most of the fluid, organic solutes, and electrolytes are reabsorbed in an isotonic fashion. The more distal nephron segments are responsible for the final regulation of electrolyte and solute absorption and secretion as well as urinary concentration and dilution. Glomerulogenesis continues until 34–36 weeks gestation. The term neonate thus has a comparable nephron endowment as an adult. The term neonate has a much lower glomerular filtration rate than an adult even when factored for body surface area. There is postnatal maturation of the glomerulus and a parallel increase in tubular resorptive capacity. Transport occurs across tubules and also across the paracellular pathway. The very premature infant is far less equipped for life outside the mother's womb. There is glomerular tubular imbalance for infants less than 34 weeks as evidenced by glucosuria and very premature infants filter far more sodium than they can recover resulting in renal salt wasting. Postnatal maturation is much more complex than originally thought. There is not only a maturational increase in transporter abundance but there are changes in isoforms of key renal transporters during development as well as change in the paracellular pathway. This chapter discusses the postnatal changes in glomerular filtration rate and tubular transport of solutes and water that occur in the term and preterm neonate.

Keywords

Renal development · Tubule transport · Glomerular filtration rate · Urinary concentration · Urine dilution · Nephron

Maturation of GFR

The neonate GFR is ~20–40 mL/min/1.73m^2 at birth in term infants and even lower in preterm infants [1–4]. GFR approximately doubles in the first 2 weeks of life in term infants but does not reach adult levels until 2 years of age [4, 5]. In infants with very low birth weight, adult level GFR may not be reached until 8 years of age [6]. Preterm infants have a sharp increase in GFR once nephrogenesis is complete after 34–36 weeks [7]. In all neonates (term and preterm), postnatal increases in GFR result from increases in renal blood flow and regulation of renal vascular resistance.

Cardiac output to the kidney increases from 3% to 10–15% in the first week to 1 month of life and then to adult levels of 20–25% by 2 years of age [7–9]. Mean arterial blood pressure also increases immediately after birth, which increases the hydrostatic pressure in glomerular capillaries. Together, increased cardiac output and increased mean arterial pressures lead to a significant increase in renal plasma flow rate in the first weeks of life. For the glomeruli to appropriately receive this increased renal flow and thus increase GFR, regulation of renal vascular resistance is crucial.

Perfusion of the glomerular capillary network of the glomerulus comes via the afferent arteriole and blood exits via the efferent arteriole. Local and systemic mediators of vasodilation and vasoconstriction act upon the afferent and/or efferent arterioles to mediate renal vascular resistance and thus glomerular perfusion and GFR. Vasodilation of the afferent arteriole and vasoconstriction of the efferent arteriole increases GFR. Conversely, vasoconstriction of the afferent arteriole and vasodilation of the efferent arteriole decreases GFR. The most important mediators leading to increase in GFR after birth are angiotensin II and prostaglandins. Angiotensin II, part of the renin-angiotensin system, acts primarily to vasoconstrict the efferent arteriole to increase glomerular pressure [10]. This helps to offset the relatively lower mean arteriole pressure of neonates to ensure adequate renal perfusion for filtration. Angiotensin II also increases systemic blood pressure via increased peripheral resistance and increases myocardial contractility to increase cardiac output [11], both of which contribute to increased renal perfusion and GFR. Prostaglandins vasodilate the arterioles. The role of prostaglandins is to offset or balance vasoconstrictive effects of hypotension, hypovolemia, and hyponatremia [12]. Both angiotensin II and prostaglandins are abundant shortly after birth [12].

Other regulators of renal arteriole vasodilation include nitric oxide, atrial natriuretic peptide (ANP), and bradykinin. Nitric oxide is a potent vasodilator that counterbalances the vasoconstrictive action of angiotensin II. Animal models show that nitric oxide synthase enzymatic activity is higher in the newborn compared to adults and plays a critical role in protecting the neonatal kidney from acute hypoxemia [13, 14]. ANP is produced by atrial myocytes in response to increased arterial volume or pressures. ANP vasodilates afferent arterioles, contributing to an increase in GFR [15]. Bradykinin is a vasodilator and its receptors are more prevalent in neonatal kidneys compared to adult kidneys [16].

Additional regulators of renal arteriole vasoconstriction are endothelin and the sympathetic nervous system. Endothelin is produced by the vascular endothelium and constricts the afferent and efferent arterioles, decreasing GFR when present at high levels. Conversely, low concentrations of endothelin present in physiologic conditions in the neonate may vasodilate the glomerular vessels [17]. Sympathetic nerves secrete norepinephrine, which constricts both afferent and efferent arterioles [18].

Adenosine vasoconstricts the afferent arteriole and vasodilates the efferent arteriole via stimulation of the angiotensin II receptor, which leads to decreased GFR if unopposed [1]. In physiologic conditions, adenosine carefully balances the efferent vasoconstrictive action of angiotensin II to maintain glomerular pressure and GFR. In pathologic conditions such as hypoxia or hypotension, excess generation of adenosine may lead to increased afferent arteriole vasoconstriction and decreased GFR [8]. Adenosine antagonists, such as theophylline or caffeine, protect against reduced GFR in term neonates with birth asphyxia [19] and in preterm neonates [20].

Autoregulation and tubuloglomerular feedback also affect vascular tone and GFR maturation [21, 22]. Autoregulation maintains constant renal blood flow despite mean arterial blood pressure fluctuations by altering arteriolar tone. Arteriole vasoconstriction in response to lower perfusion pressures seen in the neonate compared to the adult is the result of prostaglandin-dependent renin release [23]. Tubuloglomerular feedback is the means by which vascular tone responds to fluctuations in luminal sodium concentrations sensed by the macula densa and juxtaglomerular cells. For example, when blood pressure is low there is less sodium delivery so juxtaglomerular cells stimulate angiotensin II to constrict the efferent arteriole and maintain intraglomerular pressure and GFR. Adenosine counterbalances the efferent vasoconstrictive action of angiotensin II in tubuloglomerular feedback.

Overall, renal vascular resistance is elevated at birth and decreases in the first few weeks of life; this along with increased systemic blood pressure and increased cardiac output to the kidney leads to increased renal blood flow and GFR.

Measuring GFR in the Neonate

While inulin is the “gold standard” for measuring GFR in infants, it is not readily available clinically; thus, serum creatinine and cystatin C are more often used to measure GFR in neonates. Serum creatinine is the most commonly used marker of kidney function in the neonate, but has limitations. Serum creatinine reflects maternal creatinine for the first few days of life due to passive transport across the placenta and is reabsorbed by the immature tubules; thus, creatinine-based formulas can be inaccurate for neonates [24, 25]. Cystatin C has minimal transport across the placenta and a review of ten studies evaluating cystatin C GFR in neonates showed that cystatin C levels are not affected by the infant's gender, gestational age, or muscle mass [26]. Furthermore, cystatin-C based GFR equations have been validated in infants and children, including preterm infants [27, 28]. Post-conceptual age affects both cystatin C and creatinine levels, however, which should be accounted for when evaluating neonatal GFR [29].

Preterm Neonate Nephron Development and GFR

Nephrogenesis ends at 34–36 weeks gestation. The term neonate has between 200,000 and 2,000,000 nephrons per kidney [8]. The nephrons mature and hypertrophy until reaching adult size between 3 and 5 years of age [30]. In preterm infants, nephrogenesis can continue for up to 40 days after birth, so it is crucial to optimally support this process by avoiding kidney injury from nephrotoxic medications and hypotension in the first several weeks of life [12]. The number of nephrons increases by approximately 25,000 for each 100 g of birth weight [31]. Birth weight less than 1000 g was the only neonatal characteristic associated with lower eGFR in children aged 3–10 [32], suggesting that the premature, low birth weight infant is at the most risk for renal damage in the neonatal period.

Inulin clearance studies show that normally GFR increases from 28 to 36 weeks gestation, when glomerulogenesis is completed, and then plateaus until birth [2, 3, 33]. For those born preterm, GFR increases in the first week of life but not as rapidly as in the term neonate [34]. Serum creatinine also does not decrease as quickly in the first week of life in preterm infants, often taking up to 1–3 months to reach its nadir [33, 35]. In the preterm infant, GFR increases more rapidly and serum creatinine decreases more rapidly at 34–36 weeks post-conceptual age, correlating to glomerulogenesis completion [35].

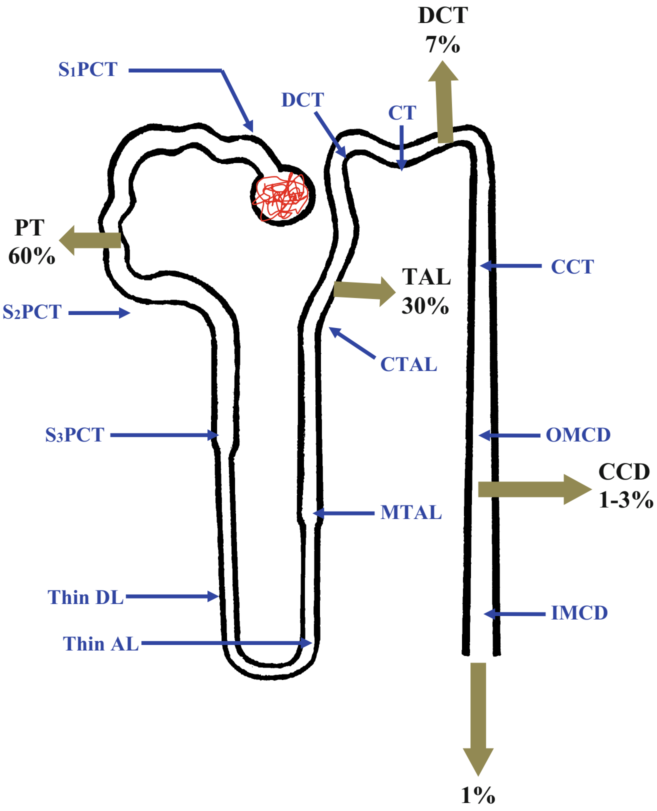
Organization of the Tubular Nephron

The nephron is faced with the task of maintaining a constant composition and volume of the extracellular fluid. The adult ingests electrolytes, nutrients, and water, and generates waste products that must be eliminated to maintain a constant composition and volume of the extracellular fluid compartment. In other words, the quantity of electrolytes that are ingested and absorbed must be eliminated and the waste products from metabolism must be also be excreted. This challenge is all the more complex as our dietary intake is quite variable from day to day. Despite this variable intake, there is virtually no change in the volume or composition of the extracellular fluid volume.

There are two possible ways that our kidney could balance ingestion and excretion. The kidney could be a secretory organ where all the excess electrolytes, solutes, and water ingested would be excreted by tubular secretion. This would be very inefficient and require an enormous amount of energy. In addition, in times of a disturbance in the extracellular fluid volume such as a high salt intake or volume loss from diarrhea, for example, the regulatory systems necessary to maintain a constant extracellular fluid volume and composition while excreting waste products would be very complex. In contrast, the kidney could filter an enormous quantity of extracellular fluid, which would be very efficient in removing waste products, and reclaim the desired salt, organic solutes, and water. With volume depletion, a greater fraction of salt and water could be reabsorbed and when one has a dietary indiscretion such as the salt intake from a pepperoni pizza, there could be a reduction in sodium reabsorption leading to an increase in excretion of salt to bring us back into balance. The mammalian kidney actually uses both mechanisms to maintain a constant composition and volume of the extracellular fluid which is necessary for survival on land. The adult kidney filters ~150 l of isotonic fluid a day and reclaims most of it, leaving the non-reabsorbed waste and salts to be excreted in about 1.5 l of urine. In addition, there are secretory processes for solutes such as organic anions and cations in the proximal tubule and mechanisms to secrete the excess acid generated from metabolism in the distal nephron which aid in maintaining homeostasis.

To accomplish the remarkable task of reclamation of the necessary solutes and water to maintain a constant composition of the extracellular fluid volume, the mammalian kidney has evolved into a highly specialized organ with thousands of units called nephrons. Each human kidney has approximately one million nephrons. Each nephron is a tube consisting of epithelial cells and is divided into 12 specialized segments as shown in Fig. 1. The epithelial cells allow for the vectorial transport of solutes. The proximal tubule is responsible for the bulk reclamation of solutes and water and for the secretion of organic cations and anions. Approximately two-thirds of the glomerular filtrate is reabsorbed by the proximal tubule in an isotonic fashion. Virtually all of the organic solutes, as well as the majority of bicarbonate, phosphate, and chloride are reabsorbed in this segment. The proximal tubule is divided into S_1, S_2, and S_3 segments based on the rates of transport of some solutes, and morphological changes that occur down the proximal tubule. The proximal tubule transitions into the thin descending limb which can be of variable length and then makes a hairpin turn into the thin ascending limb which aids in the generation of a concentrated urine. The length of the thin ascending and descending limb is variable among species with dessert rodents having very long thin limbs as they need to

Fig. 1 This cartoon depicts the nephron with its 12 segments. Shown in blue are the nephron segments. S_1, S_2, S_3 PCT depict the three segments of the proximal tubule. The loop of Henle consists of the thin descending limb (thin DL) and thin ascending limb (thin AL) and the medullary (MTAL) and cortical thick ascending limb (CTAL). The distal convoluted tubule (DCT) is comprised of the connecting tubule (CT) and the initial cortical collecting tubule. The collecting duct is made up of the cortical collecting tubule (CCT), outer medullary (OMCD), and inner medullary collecting duct (IMCD). Shown in black is the percentage of sodium reabsorbed by the proximal tubule (PT), thick ascending limb (TAL), distal convoluted tubule (DCT), and cortical collecting duct (CCD). Typically, 1% of the filtered sodium is excreted



conserve water and excrete very concentrated urine. The length of the thin ascending and descending limbs increase as one goes from the superficial cortex down to the medulla. The thin descending limb expresses aquaporin 1, a water channel, on the apical and basolateral membranes making the thin descending limb very permeable to water [36]. This segment does not actively transport solutes and has a very low solute permeability. This results in a concentrated fluid in the medulla with a very high sodium chloride content in the luminal fluid providing a passive driving force for sodium chloride diffusion out of the thin ascending limb. The thin ascending limb is impermeable to water but has a high permeability to NaCl [37]. The chloride channel (CLC-K1) in the thin ascending limb is developmentally regulated [38]. There is no expression of CLC-K1 in the fetus and in the first few days of life until the end of the first week in rats. There is a correlation between CLC-K1 and urinary osmolality suggesting an important role of this channel in generating a hypertonic medulla [38]. Diffusion of NaCl causes a high interstitial osmolality. This loop structure along with the thick ascending limb generates the countercurrent multiplication system that results in a medullary osmolality far greater than that of blood [39, 40]. The importance of passive properties of the thin limbs in this countercurrent system is exemplified in mice which are deficient in aquaporin 1 [41]. The urine osmolality of aquaporin 1 deficient mice is greater than plasma, but far less than control mice that have normal aquaporin 1 expression [41]. In addition, aquaporin 1 knockout mice cannot increase their urine osmolality in response to water deprivation unlike control mice.

The thick ascending limb is the segment responsible for ~30% of sodium chloride transport and has a vital role in generating a concentrated medulla. Apical sodium chloride absorption is mediated by the bumetanide sensitive cotransporter. One of the unique features of this segment is the fact that it is impermeable to water and thus the fluid leaving this segment is hypotonic to blood. In addition, this segment has a very high paracellular permeability to cations and is responsible for much of calcium and magnesium reabsorption. The distal convoluted tubule is responsible for ~5–10% of NaCl transport. NaCl transport in this segment is mediated by the thiazide-sensitive cotransporter. Active transcellular calcium and magnesium transport also occurs in this segment.

The rest of the distal tubule is separated into the connecting tubule and the cortical, outer, and inner medullary collecting tubule. These segments are responsible for sodium absorption, potassium secretion, final urinary acidification, and water absorption; the latter is regulated by vasopressin, which causes the insertion of aquaporin 2 water channels into the apical membrane. While the fraction of salt transport and renal acidification by the collecting tubule (or collecting duct) is a small fraction of the capabilities of some of the upstream nephron segments, the collecting tubule is responsible for the final modulation of the tubular fluid. Thus, the final composition of urine and significant regulation of transport occur in these last segments.

Principals of Membrane Transport

The cells along the nephron are quite different in the various nephron segments, which will be discussed in the subsequent sections. The cells in each nephron segment are poised for vectorial transport. The apical and basolateral membranes are, by and large, a lipid bilayer which would be impermeable to water and solutes if there were not specific proteins to facilitate transport across the apical and basolateral membranes. In addition, many transporters are regulated to adjust their rate of transport to meet the physiologic changes in volume status or concentration of solutes in the extracellular milieu.

The reabsorption of solutes along the nephron is characterized by active and passive transport processes. A typical cell (proximal tubule cell) is shown in Fig. 2. If all active transport was inhibited along the nephron, we would excrete urine with the composition and volume of the glomerular ultrafiltrate. Passive transepithelial transport is, by and large, the result of gradients generated by active transport. Most active transport is the result of the basolateral Na^+,K^+-

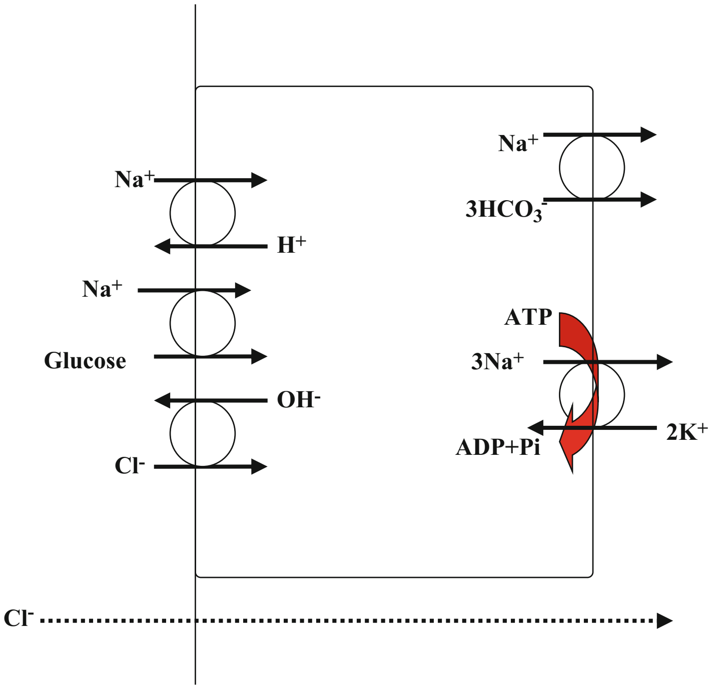


Fig. 2 A proximal tubule renal cell which shows the Na^+, K^+-ATPase on the basolateral membrane, an example of primary active transport. Na^+,K^+-ATPase decreases the intracellular sodium to about 10 mEq/l and increases the intracellular potassium to approximately 140 mEq/l. The pump is electrogenic with a cell negative potential of about 60–90 mV. The sodium gradient provides the driving force for the apical Na^+/H^+ exchanger and both the sodium gradient and the potential difference provide the driving force for the apical sodium glucose transporter. The secretion of protons via the Na^+/H^+ exchanger results in the driving force for the Cl^-/OH^- exchanger which is an example of tertiary active transport. Chloride is shown traversing the paracellular pathway. Bicarbonate is exiting the basolateral membrane via a sodium bicarbonate cotransporter

ATPase. This transporter pumps three sodiums out of the cell in exchange for two potassium ions. The pump utilizes ATP and it is an example of primary active transport. This pump is vital to the generation of the low intracellular sodium and high intracellular potassium concentration as well as the negative intracellular potential difference across the apical and basolateral membranes. Both the low intracellular sodium and this potential difference can provide a driving force for secondary active transport. For example, in Fig. 2, the reabsorption of glucose via a sodium-dependent transporter utilizes both the sodium gradient and the relative negative cell potential to bring glucose in the cell. The Na^+/H^+ exchanger on this cell is electroneutral and utilizes the sodium gradient to secrete protons and reabsorb sodium. Both the sodium glucose and the Na^+/H^+ exchanger are secondary active transport processes dependent on the basolateral Na^+,K^+-ATPase. The secretion of protons will cause the luminal pH to drop providing a favorable driving force for the Cl^-/OH^- exchanger, an example of tertiary active transport. Thus, in secondary and tertiary active transport, the transporters do not utilize ATP directly; however, inhibition of the ATP dependent Na^+,K^+-ATPase would bring these transport processes to a halt.

In addition to active transport a substantive amount of passive transport occurs between cells across the tight junction. Active transport along the nephron will generate ion and solute gradients between the lumen and peritubular fluid. Depending on the permeability properties of the tight junction, passive absorption or secretion can occur. In the cell depicted in Fig. 2, there is passive chloride transport across the paracellular pathway. It has become apparent that the characteristics of the tight junctions vary along the nephron. The tight junction creates the primary permeability barrier to diffusion of solutes across the paracellular pathway. Occludin and claudin proteins are localized to junctional fibrils and are transmembrane components of tight junctions [42–44]. These tight junction fibrils or strands are a major factor determining the permeability properties of the paracellular pathway [44–46]. The claudin family of tight junction proteins now numbers 24. Occludin has a ubiquitous distribution and is not responsible for the differential permeability properties in the various nephron segments. The claudin isoforms present at the tight junction of various epithelia determine the resistance and the permeability properties of the epithelia [44–46]. The distribution of claudin isoforms varies along the nephron and is responsible for the unique permeability properties of each nephron segment.

The final form of passive transport is called solvent drag. Solvent drag has been postulated to be responsible for a small fraction of transport in the proximal tubule. The reabsorption of solutes could result in water movement that could entrain or carry solutes with it. For this to occur, the solute would have to have a low reflection coefficient or high sieving coefficient (sieving coefficient = 1/refection coefficient). In other words, when a solute is entrained in fluid and hits a membrane or tight junction, it could pass through it and be transported across or bounce off and not be transported. Direct measurements of solute drag in the proximal tubule of neonates and adults have shown that it contributes a negligible fraction to transport [47–50].

Maturation of Na^+,K^+-ATPase Along the Nephron

The Na^+,K^+-ATPase is located on the basolateral membrane of most tubules in the kidney [51]. The Na^+,K^+-ATPase is a heterodimer composed of an α and β subunit. There are four different α subunits and three beta subunits on mammalian cells which have different functional properties [52]. There is also evidence for a small γ subunit that is not required for Na^+,K^+-ATPase activity on some cells [52, 53]. The γ subunit binds to the α subunit, stabilizes the enzyme, and plays a regulatory role in enzyme activity [52, 53]. The adult kidney expresses the α1 and ß1 isoforms of the Na^+,K^+-ATPase [54, 55]. The α subunit is the catalytic subunit and has the cation, ATP, and ouabain binding sites [52]. The β subunit is the regulatory subunit and is essential for the function of the enzyme [52, 56]. Several hormones that regulate sodium transport along the nephron act, at least in part, by regulating the Na^+,K^+-ATPase activity [52, 57–69]. Dopamine and atrial natriuretic factor inhibit, while alpha agonists and angiotensin II stimulate Na^+,K^+-ATPase activity [51]. Hormones such as dopamine directly regulate the Na^+,K^+-ATPase and do not act on transporters on the apical membrane [69].

The Na^+,K^+-ATPase is responsible for lowering the intracellular sodium concentration and establishing the negative cell potential difference. The Na^+,K^+-ATPase provides the driving force for sodium transport across the nephron. As shown in Fig. 3, there is a direct relationship between sodium transport and Na^+,K^+-ATPase activity factored per millimeter of tubule along

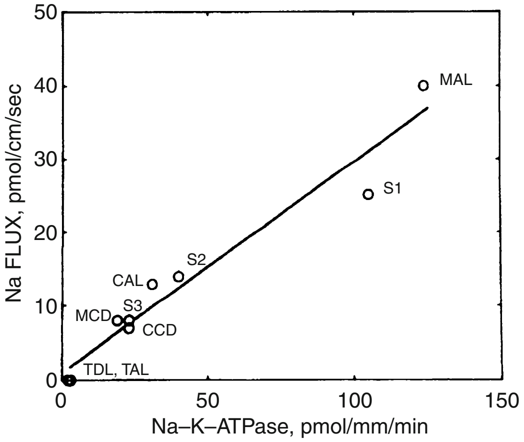


Fig. 3 Sodium transport is plotted against Na^+,K^+-ATPase activity. As is shown the rate of sodium transport in various nephron segments parallels Na^+,K^+-ATPase activity. *CAL* cortical thick ascending limb, *CCD* cortical collecting duct, *MAL* medullary thick ascending limb, *MCD* medullary collecting duct, *S1–3* S1–3 segments of the proximal tubule, *TAL* think ascending limb, *TDL* thin descending limb

the nephron [70]. Indeed, an increase in intracellular sodium concentration increases Na^+,K^+-ATPase activity [71]. Neonates have a lower renal Na^+,K^+-ATPase activity than do adult kidneys [68, 72–76]. As will be discussed, there is a developmental increase in sodium transport in each nephron segment, which is paralleled by an increase in Na^+,K^+-ATPase activity as shown in Fig. 4.

The parallel maturational increase in sodium transport with Na^+,K^+-ATPase activity [73] along with the striking relationship between sodium transport and Na^+,K^+-ATPase activity [70], suggests that the maturational increase in apical sodium transport may contribute to the postnatal increase in Na^+,K^+-ATPase. In cell culture studies, an increase in intracellular sodium resulted in a stimulation in Na^+,K^+-ATPase activity [77, 78] as well as an increase in the α subunit mRNA and membrane Na^+,K^+-ATPase density on the basolateral membrane [79]. In addition to in vitro studies, there is evidence that a chronic increase in Na^+/H^+ exchanger activity induced by metabolic acidosis, which could result in an increase in intracellular sodium, stimulated basolateral Na^+,K^+-ATPase activity, an effect that was blocked by coadministration of the Na^+/H^+ exchange inhibitor, amiloride [80]. Finally, there is a postnatal increase in both serum thyroid hormone and glucocorticoid levels with age [81–84]. Both hormones have been shown to increase Na^+,K^+-ATPase activity [58, 76, 85–88]. An in-depth discussion of the developmental expression, characteristics, and regulation of the Na^+,K^+-ATPase was presented in the Homer Smith award to Dr. Anita Aperia [6].

Proximal Tubule Transport

Proximal tubule transport is characterized by a phenomenon called threshold, which is depicted on Fig. 5. It is the threshold that keeps our serum bicarbonate at 25 mEq/L. If we were to ingest bicarbonate and try to raise our serum bicarbonate level, we would have a bicarbonaturia and our serum levels would return to 25 mEq/L as long as we were euvolemic. Our serum glucose is set by other factors well below the threshold level. As shown in Fig. 5, if we increased the serum glucose level, we would reabsorb more glucose until the load of glucose delivered to the proximal tubule exceeded its ability to reabsorb glucose and we would have glucosuria.

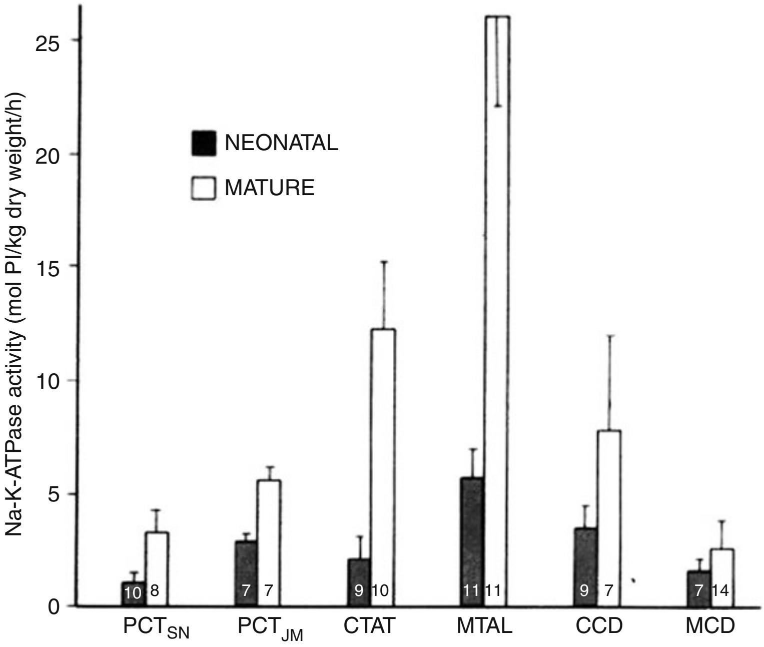


Fig. 4 Na^+,K^+-ATPase activity is shown in nephron segments of neonates and adults. As is demonstrated, there is a maturational increase in Na^+,K^+-ATPase activity in every nephron segment. PCT_{SN} proximal convoluted tubule from superficial nephrons, PCT_{JM} proximal convoluted tubule from juxtamedullary nephrons, *CTAL* cortical thick ascending limb, *MTAL* medullary thick ascending limb, *CCD* cortical collecting duct, *MCD* medullary collecting duct

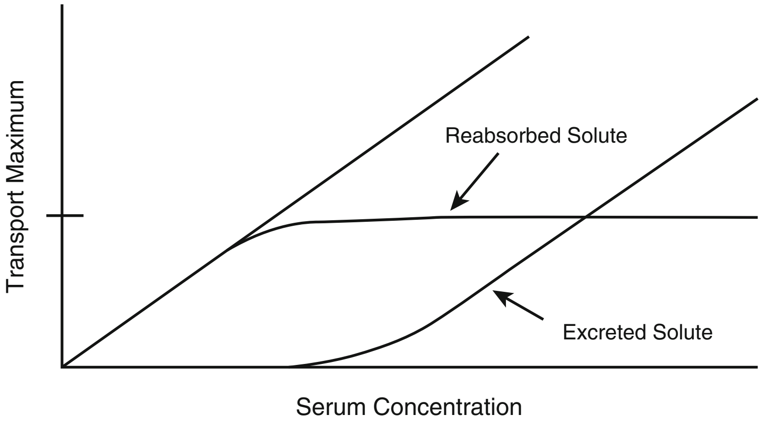


Fig. 5 Diagram depicting the concept of renal threshold. As the delivered solute load increases either by an increase in the serum concentration or an increase in glomerular filtration rate, the amount of solute absorbed increases. At some point, the renal tubular absorption reaches a maximum, called the threshold for that solute, and any further increase in the filtered load is excreted

In the adult kidney, there is a parallel change in proximal tubule transport with alterations in glomerular filtration rate. This phenomenon has been designated glomerular tubular balance. If the rate of proximal tubular transport was fixed, an increase in glomerular filtration rate would swamp the distal nephron with solutes and water and there would be a huge natriuresis and diuresis. A similar phenomenon must occur during postnatal development where there is a developmental increase in glomerular filtration rate. If there was not a parallel increase in proximal tubule transport with the maturational increase in glomerular filtration rate, the neonate would die of dehydration when the glomerular filtration rate increased after birth.

While during postnatal development there is a concomitant increase in tubular transport to accommodate or balance the increase in glomerular filtration rate [89–91], glomerular tubular balance is not present in the fetus [92]. Renal development is characterized by centrifugal maturation. The surface nephrons are relatively immature compared to the juxtamedullary nephrons. These immature nephrons with short proximal tubules have glomerular tubular imbalance [92]. This is clinically relevant as neonates born before 34 weeks gestation can have glucosuria and very premature neonates can have significant renal salt wasting [93].

The proximal tubule reabsorbs 60% of the glomerular filtrate in an isosmotic fashion. Due to the fact that the proximal tubule has a relatively high permeability to many ions, even solutes which are not actively transported by this segment may be absorbed by diffusion across the paracellular pathway. For example, since the proximal tubule reabsorbs over half of the glomerular filtrate, the luminal concentration of a solute such as magnesium, which is not actively transported by this segment, would increase over twofold. However, this does not happen because as the magnesium concentration rises above that in the peritubular capillaries, magnesium is passively reabsorbed across the paracellular pathway down the concentration gradient generated by the reabsorption of other solutes.

Glucose Transport

Glucose is reabsorbed solely by the proximal tubule (see ▶ Chap. 39, "Aminoaciduria and Glycosuria in Children"). Physiologic studies have demonstrated that the S_1 proximal tubule reabsorbs glucose by a high capacity, low affinity transporter, while in the late proximal tubule (S_3), glucose transport is via a low capacity, high affinity transporter [94]. Similar axial heterogeneity of glucose transport kinetics was validated using cortical brush border membrane vesicles to measure apical membrane transport and outer medullary brush border membrane vesicles which contain vesicles from the S_3 segment [95]. The high capacity, low affinity sodium-dependent glucose transporter on the apical membrane is designated SGLT-2 [96]. This transporter removes the bulk of the glucose from the glomerular ultrafiltrate. The low capacity, high affinity transporter is designated SGLT-1 and reabsorbs the glucose not reabsorbed in the early proximal tubule [97, 98]. The glucose that is transported into the tubule exits across the basolateral membrane by facilitative diffusion via GLUT2. Mutations in GLUT2 lead to Fanconi-Bickel syndrome [99, 100]. As shown in Fig. 6, SGLT-2 transports one sodium with one glucose molecule while SGLT-1 transports two sodium with each glucose molecule. A defect in SGLT-1 causes glucose-galactose malabsorption as this transporter is also present in the intestine [101–104]. Some patients with familial glucosuria, a benign condition, have a mutation in SGLT-2 [105, 106]. This axial arrangement of glucose transporters, where there is a high capacity, low affinity transporter followed by a low capacity, high affinity glucose transporter, results in reabsorption of virtually all of the filtered glucose.

Sodium-dependent glucose reabsorption results in a positive charge entering the proximal tubule cell. This charge leaves a lumen negative trans-epithelial potential difference. This negative potential provides a driving force for the absorption of an anion (chloride) or the back diffusion of a cation (sodium) across the paracellular pathway. Thus, glucose transport can result in a net absorption of sodium chloride with sodium moving into the cell with glucose and chloride across the paracellular pathway. Whether sodium is recycled back into the lumen or chloride is reabsorbed across the paracellular pathway is dependent on the relative sodium/chloride permeability of the paracellular pathway.

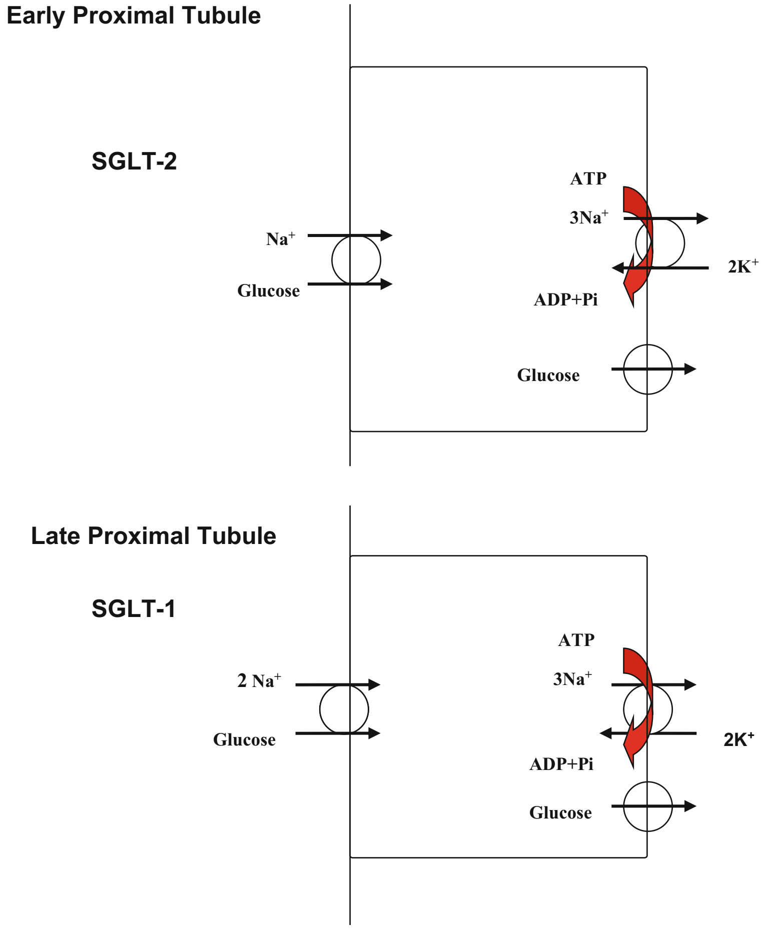


Fig. 6 Diagram of glucose transport in the proximal tubule. The early proximal tubule has SGLT-2 on the apical membrane which is a high capacity, low affinity transporter. The late proximal tubule has a low capacity, high affinity transporter designated SGLT1 on the apical membrane. Glucose exits the cell across the basolateral membrane by passive diffusion

Numerous studies using various techniques and animal species have shown that the fetus and neonate transport glucose at a slower rate than the adult [92, 107–110]. These studies are of clinical relevance as premature neonates can have glucosuria [93, 111, 112]. Despite the fact that the glomerular filtration rate of a premature neonate is about 1% of that of an adult and thus the filtered load delivered to the neonatal proximal tubule is also about 1% of that of the adult, the filtered load can exceed the resorptive capacity for glucose transport in the premature neonate. Thus, there is a time during development where glomerular tubular balance is not present. Kinetic studies demonstrate that the Km (the glucose concentration at half maximal velocity) for the glucose transporter is comparable between the fetus and adult, however the Vmax (maximal rate of glucose transport) is much lower in the neonate [113]. This indicates that the developmental increase in transport is not due to a glucose transporter isoform change but due to a maturational increase in the number of glucose transporters on the apical membrane.

Amino Acid Transport

All renal amino acid transport occurs in the proximal tubule (see ▶ Chap. 39, “Aminoaciduria and Glycosuria in Children”). Unlike most cells that have amino acid transporters to provide substrates for protein synthesis, the proximal tubule mediates the vectorial transport of amino acids from the filtrate to the blood. The basic principal for transport is similar to glucose transport. The uptake of amino acids is sodium dependent and electrogenic with the basolateral exit mediated by facilitated passive diffusion. While there are 20 amino acids that are utilized in the synthesis of proteins, there are not 20 different amino acid transporters on the apical and basolateral membranes. Since some amino acids are similar in structure and/or charge, there is promiscuity among the classes of amino acid transporters. We will briefly discuss the three major classes of amino acid transporters.

The neutral amino acids include leucine, valine, isoleucine, methionine, phenylalanine, tyrosine, cysteine, glutamine, alanine, glycine, serine, histidine, tryptophan, and proline. Transport of these amino acids is electrogenic with one sodium ion being transported with an amino acid across the apical membrane. B^0AT1 (SLC6A19) and is expressed on the proximal tubule [114–116] and transports all neutral amino acids, although there is greater affinity for valine, leucine, isoleucine, and methionine [117]. Mutation of SLC6A19 causes Hartnup disease which is an autosomal recessive disorder of variable severity characterized by a pellagra-like rash, cerebellar ataxia psychological and neurological disturbances [115, 116, 118]. The expression of B^0AT1 on the apical membrane is dependent on a protein called collectrin (Tmem27) [119]. A mutation and loss of collectrin also results in a Hartnup phenotype [120]. The symptoms due to Hartnup disorder are likely the result of impaired intestinal and renal transport of tryptophan [121]. There are other neutral amino acid transporters but their transport properties have been less well characterized [118, 122].

The acidic amino acids are aspartate and glutamate. They are transported across the apical membrane of the proximal tubule in an electrogenic fashion where two sodium ions are transported for each of these negatively charged amino acids [123, 124]. Brush border membrane vesical studies have shown that there are at least two apical transporters for glutamate, one with a high substrate affinity and one with a low affinity [125]. The high affinity transporter has been cloned and designated SLC1A1 (EAAC1) [126]. SLC1A1 is expressed on the apical membrane of the proximal tubule [127]. *Slc1a1* knockout mice have a dicarboxylic aciduria proving the importance of this transporter in acidic amino acid transport [128]. Recently, patients with SLC1A1 mutations have been found to have dicarboxylic aminoaciduria and mental retardation likely due to the importance of SLC1A1 for glutamate transport in the brain [129]. The basolateral transport of glutamate is via a sodium-dependent cotransporter indicating that the intracellular glutamate levels must be very high in the proximal tubule to provide an adequate driving force for sodium exit across the basolateral membrane [130]. In addition, there is a sodium independent aspartate/glutamate transporter which is localized to the basolateral membrane designated AGT1 [131].

The basic amino acids lysine and arginine utilize the same amino acid transporter as cystine and ornithine (COLA). Cystine, lysine, arginine, and ornithine are transported across the apical membrane of the proximal tubule by a heterodimer of two proteins designated rBAT (SLC3A1) and b^{o+}AT (SLC7A9). rBAT is a cystine/dibasic amino acid transporter expressed along the proximal tubule but predominantly in the S_3 segment [132, 133]. Mutations in rBAT (SLC3A1) results in type I cystinuria [134–137]. Mutations in B^{0+}AT which is also a cystine transporter/dibasic amino acid transporter causes type II cystinuria [138, 139]. While there is increased excretion of cystine, arginine, lysine, and ornithine in patients with cystinuria, cystine is by far the least soluble leading to recurrent kidney stones. Type I cystinuria is inherited as an autosomal recessive disorder, while the inheritance of type II is cystinuria is less clear but likely an autosomal dominant disorder with incomplete penetrance [140, 141]. Patients with SLC7A9 mutations may have a milder and later in onset of stone formation and may only develop stones if volume depleted and they consume a high protein diet [140, 142]. Unlike rBAT, B^{0+}AT is predominantly expressed in the early proximal convoluted tubule, although it overlaps with the expression of rBAT [133, 143]. While both rBAT and B^{0+}AT can function as cystine/dibasic amino acid transporters, they likely function in vivo as a heterodimer [144]. rBAT has been shown to be developmentally regulated with no or very low expression in neonatal rats before weaning [145]. There is an increase in rBat expression on the brush border membrane at the time of weaning but to only $<$15% of the adult level [145].

Neonates have a generalized aminoaciduria, which is more pronounced in premature neonates [146–148]. Infants have also been shown to have a higher urine concentration of most amino acids compared to young children [145, 146]. While there have been numerous studies examining amino acid transport using a number of species, most have utilized kidney slices or slurries of renal tubules. These studies are complicated by the fact that one is looking simultaneously at cellular uptake, metabolism of the amino acid, and basolateral exit (often from a collapsed tubule). Examination of glycine transport in tubule suspensions and kidney slices of neonates and adults have provided disparate results. Glycine uptake has been shown to be the same in neonates and adults in some studies [149, 150] and lower in neonates than adults in others [151, 152]. The few studies looking at brush border membrane during development, a more direct way of studying uptake transport across the apical membrane, have shown lower rates of amino acid transport in the neonate compared to the adult [153–155].

Organic Anion and Cation Transport

In addition to filtration and reclamation, the kidney has the ability to secrete some substances through organic anion and cation transporters designated OATs and OCTs. There are multiple organic anion transporters on the basolateral membrane of the proximal tubule with different substrate specificities [156–159]. Organic acids transported by OATs include prostaglandins, uric acid, nonsteroidal anti-inflammatory drugs, β lactam antibiotics, antiviral medications, para-amino hippurate, probenecid, diuretics, salicylates, methotrexate, and many others [157–159]. Many of these substances are protein bound in the blood which limits their excretion by glomerular filtration. Secretion of organic anions is an example of tertiary active transport [158, 159]. For example, organic anions are taken up into the cell predominantly in exchange for α-ketoglutarate which enters the cell via a sodium dicarboxylate cotransporter. The energy for this whole process is the basolateral Na^+,K^+-ATPase. The organic acid transported inside the cell must exit across the apical membrane to enter the urine. The mechanism for this includes members of the multidrug resistance protein family as well as other organic anion secretory exchangers [157–160]. The organic cation excretion is via an organic cation transporter on the basolateral membrane down its electrochemical gradient with secretion into the lumen can be via a number of different mechanisms including in exchange for a proton or via a multidrug resistant protein [160].

Para-amino hippurate is almost totally removed from the blood with one pass through the kidney and can be used as a measure of renal blood flow. Para-amino hippurate is primarily transported across the basolateral membrane of the proximal tubule via OAT1 [161, 162]. Para-amino hippurate has been used to assess the maturation of renal blood flow in humans and to determine the maturational changes in organic anion transport. Studies in humans have shown that there is a maturational increase in para-amino hippurate secretion with adult values being attained at about 2 years of age [163, 164]. Para-amino hippurate secretion is less in premature than term neonates [3].

There are a number of factors that could contribute to the maturational increase in organic anion secretion. Organic anion secretion requires an organic anion transporter, a sodium-dependent organic acid cotransporter, and the Na^+,K^+-ATPase to mediate intracellular transport of the organic acid and an apical secretory mechanism to secrete the organic acid; thus, a paucity in any of these transporters in neonates, compared to adults, could be the rate limiting step. While OAT1, OAT2, OAT3, and OAT5 have been shown to be present in the late gestation fetus, expression of each of these OATs increases during postnatal development [162, 165–168]. There is also a maturational increase in ATP-binding cassette (ABC) transporters, which also are involved in drug secretion [162].

One of the unique features of organic anion transport is that it can be induced prematurely during renal development by an organic anions [169–172]. This is not true of adult animals where organic anions do not cause a stimulation in transport [170]. In vitro microperfusion studies have demonstrated intrinsic increases in the rate of transport with postnatal age in rabbits and that pretreatment with penicillin in vivo increased the rate of para-amino hippurate secretion in isolated proximal straight tubule perfused in vitro [169]. The mechanism of this induction of organic anion transport by organic acids is unclear.

Phosphate Transport

The adult ingests approximately 1–1.5 g of phosphorus per day and of that ~80% is absorbed. The adult must be in neutral phosphorus balance, meaning that the amount of phosphorus absorbed from day to day has to equal that excreted. The phosphorus in our body is predominantly in the form of phosphate. At a pH of 7.4, there is a 4:1 ratio of HPO_4^{-2}/ $H_2PO_4^{-1}$. The kidney maintains phosphate balance by the ability to regulate phosphate transport, which predominantly occurs in the proximal tubule (see also ▶ Chap. 40, “Renal Hypophosphatemia”). The main factors that regulate renal phosphate transport are dietary intake and hormones including parathyroid hormone, FGF-23, and growth hormone. Phosphate is essential for bone growth and 85% of our phosphate is in bones. In addition, phosphate is involved in a myriad of enzymatic reactions and is present in nucleotides, phospholipids, and proteins. Unlike the adult, the neonate must be in positive phosphate balance for growth. This section will review phosphate transport and then discuss developmental changes which occur in transport and its regulation which allow the neonate to be in positive phosphate balance.

The transporters involved in the regulation of phosphate transport are shown in Fig. 7. The first phosphate transporter cloned, designated NaPi-1, did not have the characteristics previously identified in physiologic studies and its function is still not clear [173, 174]. There are two sodium-dependent phosphate transporters on the apical membrane of the proximal tubule, one designated NaPi-IIa [175] and the other NaPi-IIc [176]. NaPi-IIa is an electrogenic transporter that transports three sodium ions with one phosphate (HPO_4^{-2}), while NaPi-IIc transports two sodium ions with every phosphate and is electroneutral [176, 177]. NaPi-IIa and Napi-IIc are regulated by PTH and dietary phosphate intake [178–181]. Phosphate exits the proximal tubule by a transporter which has not yet been identified and characterized. NaPi-IIb is the phosphate transporter on the intestinal apical membrane responsible for absorption of dietary phosphate [182].

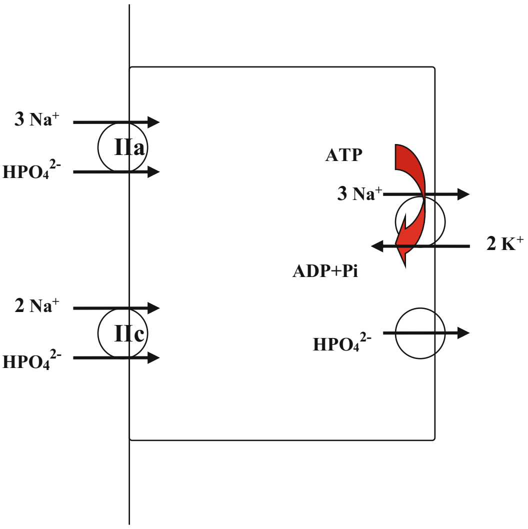


Fig. 7 Diagram of phosphate transport in the proximal tubule. The apical phosphate transporter NaPi-IIa (top) is the predominant phosphate transporter in adult rodents. NaPi-IIc (bottom) is the predominant phosphate transporter on the apical membrane of neonatal rodents. NaPi-IIa is electrogenic while NaPi-IIc is electroneutral. NaPi-IIc is the main phosphate transporter on the apical membrane of the proximal tubule in humans

The serum phosphate levels are higher in neonates than adults [183, 184]. Since the glomerular filtration rate in the neonate is only a fraction that of the adult, a low GFR could theoretically be responsible for the relative hyperphosphatemia in neonates. However, studies in humans found that the fraction of phosphate reabsorbed compared to the glomerular filtration rate was higher in neonates and infants than that in adults [183–186]. In the first 24 h of life, human neonates reabsorb over 95% of the filtered phosphate [183]. This level drops to 90% by about week of age [183, 184]. This fractional reabsorption of phosphate in neonates meets or exceeds the resorptive capacity of adults and older children eating a normal phosphate diet [186].

As seen with humans, young rats have a greater rate of renal phosphate reabsorption of than adult rats [187]. An increase in phosphate transport was also found in rats that received a parathyroidectomy indicating that a blunted response to parathyroid hormone could not explain the higher rates of phosphate transport in neonates compared to adults [187]. Finally, while both young and adult rats responded to a low phosphate diet with an increase in the fractional reabsorption of phosphate, the magnitude of renal phosphate absorption was again higher in young animals [187].

While the above studies are consistent with an enhanced tubular resorptive capacity in neonates, this could be due to a higher resorptive capacity of the neonatal tubule, a diminished response to a phosphaturic factor(s) or the result of an increased response to a substance which increases phosphate transport. To determine if there was an inherent increase in tubular phosphate transport, Johnson and Spitzer examined phosphate absorption in neonatal and adult kidneys perfused in vitro [188]. As shown in Fig. 8, neonatal kidneys had a higher phosphate resorptive rate at any filtered load of phosphate which can only be due to an increased inherent rate of renal phosphate reabsorption. In addition, they found that while addition of parathyroid hormone to the perfusate caused a phosphaturia in adult kidneys, there was no increase in phosphate excretion in neonatal kidneys [188]. These studies directly demonstrate that neonates also have an attenuated response of one of the primary factors regulating phosphate transport, parathyroid hormone. A blunted affect of parathyroid hormone on phosphate reabsorption has also been demonstrated in young rats compared to adult rats in vivo [189, 190].

Micropuncture studies of neonatal and adult guinea pigs and rats have also demonstrated a higher intrinsic rate of proximal tubule phosphate transport in young animals [191, 192]. Studies using brush border membrane vesicles have found that the maximal rate (Vmax) of phosphate transport was several fold higher in neonates than adults, while there was no difference in the Km, the phosphate concentration at half maximal velocity [193]. A low phosphate diet increased the Vmax of the sodium phosphate transporter in adult guinea pigs while a high phosphate diet had the opposite effect. However, there was no significant difference in Vmax for phosphate uptake in brush border membranes from neonates gavaged with a low phosphate diet despite an ~50% decrease in serum phosphate [193]. A high phosphate diet in neonates resulted in a doubling of the

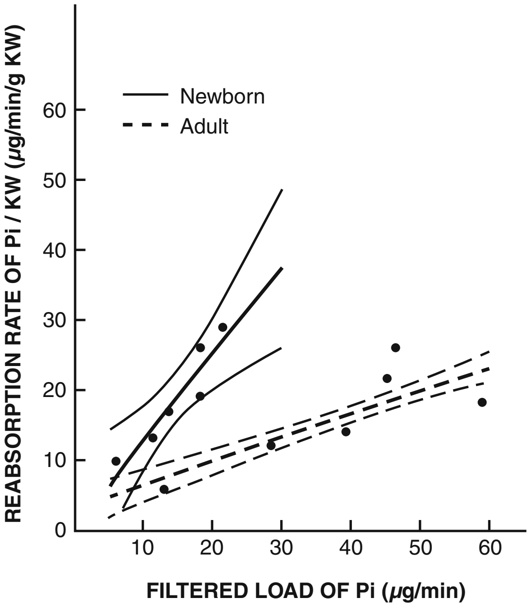


Fig. 8 The rate of phosphate absorption by isolated perfused kidney preparation. As the filtered load increases, there was an increase in the resorptive rate in both the neonate and the adult kidney. At all filtered loads, the rate of phosphate reabsorption per gram of kidney weight was higher in the neonate. (From Ref. [188], with permission)

serum phosphate, while there was little change in serum phosphate in adults. However, brush border membrane vesicles from adults on a high phosphate diet showed a more marked decrease in brush border membrane phosphate transport compared to neonates [193]. Thus while dietary phosphate and serum phosphate levels are important regulators of proximal tubule phosphate transport in adults, phosphate transport is rather fixed in neonatal guinea pigs and not significantly regulated by serum phosphorus levels or dietary phosphate intake. As shown in Fig. 9, 3-week-old rats have a maximal rate of phosphate absorption that was higher than that of adult rats [194]. In young and adult rats, there was an augmentation in the maximal capacity of phosphate reabsorption with a low phosphate diet [194]; however, there was a greater effect of phosphate deprivation on brush border membrane vesicle phosphate uptake and NaP-IIa protein abundance in 4-week-old rats compared to older adult rats [192]. Thus, while there may be a species difference, it appears that the ability to respond appropriately to a low dietary phosphate intake with an appropriate increase in phosphate transport occurs after weaning and is greater in weanling rats than in adults [192]. Finally, the driving force for phosphate entry across the apical membrane may be greater in neonates.

Maturational studies examining the changes in NaPi-IIa expression have revealed that NaPi-IIa mRNA and protein are not detected in developing nephrons until there is a distinct brush border membrane [195]. NaPi-IIa protein abundance was greater in brush border membranes from 13-day-old rats compared to 22-day-old rats [195]. Others, however, have found that brush border membrane vesicles from suckling and adult rats had a slower rate of phosphate uptake than weanling rats (21-day old) [196]. There was no change in NaPi-IIa mRNA abundance, but studies in brush border membrane vesicles revealed that 21-day-old rats had the highest NaPi-IIa protein expression [196]. Studies comparing 28-day-old rats to adult rats have also demonstrated greater brush border membrane NaPi-IIa protein abundance in young rats than adults [192]. The higher rate of renal phosphate transport is likely the major reason for the higher serum phosphate levels in neonates and weanling rats compared to adults. The high rates of phosphate transport in weanling rodents suggested that there may be a different transporter with greater expression during early stages of development [197]. Indeed, NaPi-IIc, another phosphate transporter on the apical membrane of the proximal tubule, has its highest expression rodents at the time of weaning [176]. NaPi-IIc, like NaPi-IIa, is regulated by dietary phosphate uptake and PTH [176, 198]. The relative importance of NaPi-IIa and NaPi-IIc may be quite different in humans. Patients with hereditary hypophosphatemic rickets with hypercalciuria, a rare autosomal recessive characterized by hypophosphatemia secondary to renal phosphate wasting and high vitamin D levels, have a mutation in the gene encoding NaPi-IIc indicating that NaPi-IIc is the predominant renal phosphate transporter in humans and that NaPi-IIa cannot compensate for the loss of NaPi-IIc [199].

Growth hormone increases phosphate transport via stimulation of IGF-1 in the proximal tubule [200]. Brush border membrane vesicle

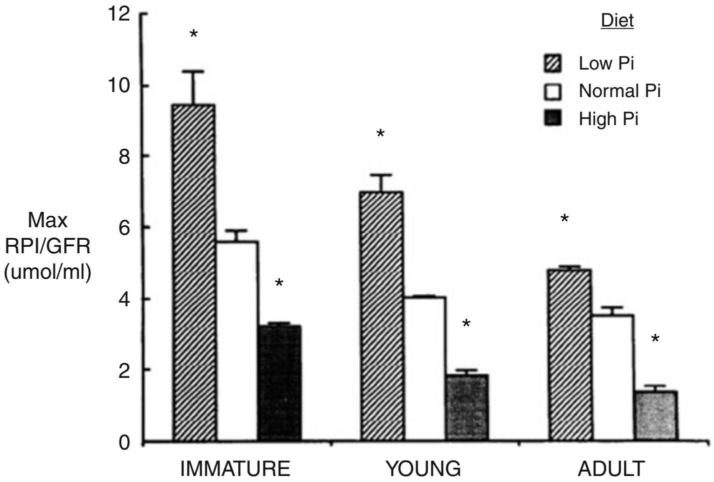


Fig. 9 Age-dependent maximal phosphate reabsorption rats at 3–4 weeks (immature), 6–7 weeks (young), and 12–13 weeks of age (adult). All rats were parathyroidectomized. A low dietary phosphate intake stimulated phosphate absorption in all age groups. The maximal capacity for phosphate absorption is found in the immature groups which need phosphate for growth. (From Ref. [194], with permission)

phosphate transport is increased in dogs that were administered growth hormone compared to vehicle treated controls [201]. While growth hormone is not a significant regulator of phosphate transport in the adult, this may not be the case in the growing animal. Administration of a growth hormone-releasing factor antagonist, which suppresses growth hormone secretion, has no effect on phosphate transport in adult rats, but significantly reduces phosphate absorption in young growing rats [202–204].

There are other hormones that have been shown to regulate phosphate transport including insulin [205], fibroblast growth factor-23 [206–208], frizzled-related protein 4 [209, 210], and Klotho [211, 212]. These hormones may have differential effects on phosphate transport in the neonate and adult, but this is yet to be determined.

Proximal Tubule Acidification

The proximal tubule reabsorbs 80% of the filtered bicarbonate. Luminal proton secretion occurs via the Na^+/H^+ exchanger and the H^+-ATPase. In the adult, one-third of proton secretion is via the luminal H^+-ATPase, and two-thirds is mediated by the luminal Na^+/H^+ exchanger that is designated NHE3 [213, 214]. The secreted proton titrates the filtered HCO_3^- to generate H_2CO_3 which is converted to CO_2 and H_2O by luminal carbonic anhydrase (Carbonic anhydrase IV). CO_2 diffuses into the cell and combines with H_2O which is facilitated by intracellular carbonic anhydrase (Carbonic anhydrase II) to regenerate H_2CO_3. H_2CO_3 dissociates into a proton that can be secreted across the apical membrane, and bicarbonate that exits the basolateral membrane via the basolateral $Na(HCO_3)_3$ symporter. The sodium which enters the call via the Na^+/H^+ exchanger exits the basolateral membrane by the Na^+,K^+-ATPase, which provides the driving force for luminal proton secretion by the Na^+/H^+ exchanger. There are maturational changes in most of these processes that will be described below.

Neonates have a lower serum bicarbonate concentration than adults, which is the result of a lower threshold for bicarbonate [215]. The threshold is the serum level where all of the filtered solute can be reabsorbed. If the concentration in the plasma exceeds the threshold, there will be more filtered solute than can be reabsorbed and the excess will be excreted into the urine until the plasma level is once again at the threshold level (see Fig. 5). Premature neonates can have physiologic bicarbonate concentrations as low as 15 mEq/l [216]. The lower bicarbonate threshold is mediated in large part by the lower rate of proximal tubule acidification. The fine tuning of renal acidification is mediated in the distal nephron which, by and large, is responsible for the secretion of acid from metabolism and new bone formation. Studies have shown that there is a maturational increase in bicarbonate absorption during postnatal development [108, 217], which accounts for the mutational increase in the bicarbonate threshold.

The greatest developmental changes in proximal tubule acidification occur on the apical membrane. There is a fourfold increase in Na$^+$/H$^+$ exchanger activity during postnatal maturation and an even greater increase in apical H$^+$-ATPase activity [213, 218, 219]. Low levels of Na$^+$/H$^+$ exchanger activity have been measured in the fetus as well [220]. While 1–2-week-old neonatal rats have Na$^+$/H$^+$ exchanger activity on the apical membrane, as shown in Fig. 10, virtually no NHE3 exists on the neonatal brush border membrane [219, 221]; in contrast, NHE3 is the primary Na$^+$/H$^+$ exchanger found on the apical membrane of the adult proximal tubule [222, 223].

Surprisingly, NHE3 knockout mice maintain some proximal tubule apical membrane Na$^+$/H$^+$ exchanger activity [224]. The apical Na$^+$/H$^+$ exchanger likely responsible for this non-NHE3 Na$^+$/H$^+$ exchanger activity is NHE8, a neonatal NHE isoform [225, 226]. NHE8 has sodium-dependent proton extrusion capabilities, which made it a potential candidate for the developmental isoform [227]. As shown in Fig. 10, apical NHE8 is predominantly present in neonatal proximal tubules at a time when NHE3 was almost undetectable [221, 228]. Similar to NHE3 in adults [223, 229, 230], NHE8 activity increases with metabolic acidosis [228, 230, 231]. The developmental isoform switch is likely mediated by the increase in thyroid hormone and glucocorticoids that occur at the time of birth through weaning [232, 233].

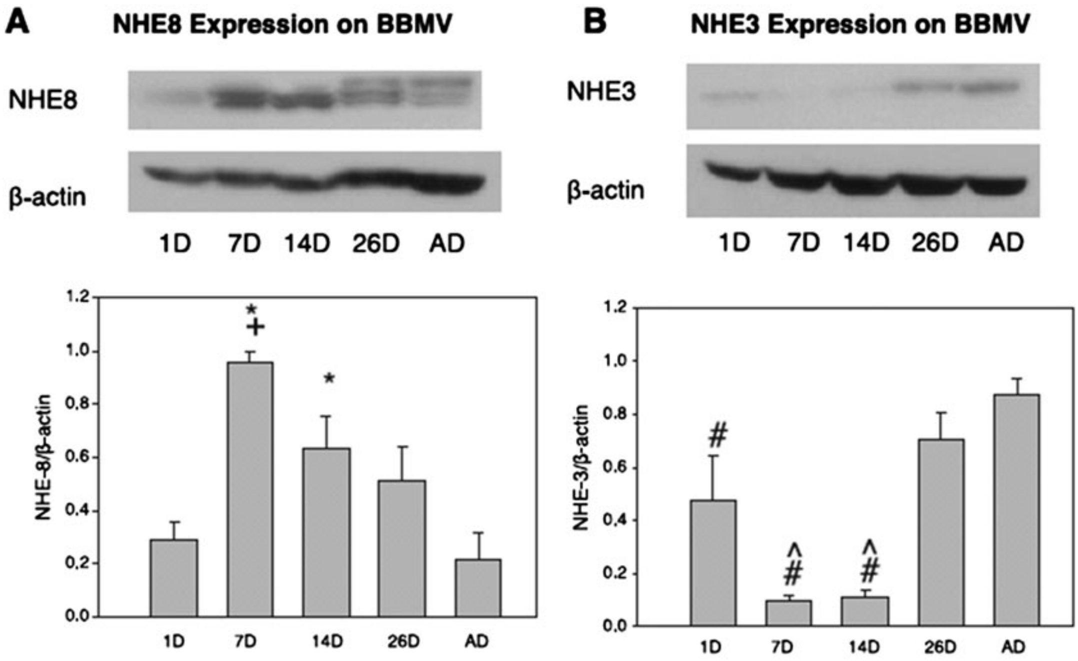


Fig. 10 Immunoblots of rat brush border membrane vesicles depict age-related changes in NHE8 (**a**) and NHE3 (**b**) protein abundance. As is seen, there is higher expression of NHE8 in the neonate than in the adult on brush border membrane vesicles (BBMV). The expression of NHE3 is highest in the adult. The higher NHE3 protein abundance at 1 day of age is likely the result of glucocorticoids at the time of birth. (From Ref. [221], with permission). *D* day, *AD* adult

The $Na(HCO_3)_3$ symporter mediates bicarbonate exit in both the neonatal and adult proximal tubule. While there is a maturational increase in basolateral membrane $Na(HCO_3)_3$ symporter activity, it is relatively small compared to the magnitude of the change in Na^+/H^+ exchanger activity on the apical membrane [234]. In addition to bicarbonate exit, the basolateral membrane $Na(HCO_3)_3$ symporter plays an important role in pH regulation of the proximal tubule cell [234, 235].

Carbonic anhydrase increases the rate of the interconversion of CO_2 and H_2O to H_2CO_3. Carbonic anhydrase II is located intracellularly in proximal and distal tubule acidifying cells and comprises ~95% of cell carbonic anhydrase activity. Carbonic anhydrase IV is on the apical and basolateral membrane of renal acidifying cells and comprises ~5% of carbonic anhydrase activity [236]. Both carbonic anhydrase II and IV increase during maturation of proximal and distal acidification, although neither is likely a limiting factor causing the maturational increase in renal acidification in the proximal or distal tubule [237, 238].

Since the developmental increase in renal acidification is due primarily to apical proton secretion, many studies have examined the cause for the increase in Na^+/H^+ exchanger activity during postnatal development. There is a substantive increase in both thyroid hormone and glucocorticoids at the time of weaning and both hormones increase in parallel with the increase in proximal tubule Na^+/H^+ exchanger activity [81–84]. Administration of either glucocorticoids or thyroid hormone prior to the normal maturational increase in either hormone results in a premature increase in exchanger Na^+/H^+ activity and NHE3 protein abundance [81, 217, 239]. Both thyroid hormone and glucocorticoids increase NHE3 activity by increasing transcription [240, 241]. Glucocorticoids have also recently been shown to increase the insertion of NHE3 into the apical membrane of proximal tubular cells by a posttranscriptional mechanism [242]. Interestingly, neither prevention of the maturational increase in glucocorticoids or thyroid hormone alone can totally prevent the postnatal increase in Na^+/H^+ exchanger activity and NHE3 mRNA and protein abundance [81, 217, 243, 244]. Thus, there appears to be some redundancy in the postnatal maturational triggers for NHE3. As demonstrated in Fig. 11, prevention of the maturational increase in both hormones results in the total prevention of the postnatal increase in NHE3 mRNA, protein abundance and Na^+/H^+ exchanger activity [245]. Both glucocorticoids and thyroid hormone have also been shown to decrease the expression of NHE8 providing further evidence that thyroid hormone and glucocorticoids are the principle factors responsible for the developmental isoform switch that occurs at weaning [232, 233].

Proximal Tubule NaCl Transport

The proximal tubule receives the glomerular ultrafiltrate and changes composition of the tubular fluid in an isosmotic fashion. The early proximal tubule reabsorbs glucose, amino acids, and bicarbonate in preference to chloride [246, 247]. The reabsorption of sodium with glucose and amino acids results in a lumen negative potential difference (due to increased lumenal chloride). The luminal fluid in the late proximal tubule has a higher chloride and lower bicarbonate concentration than that of the peritubular fluid. The lumen to peritubular chloride gradient provides a driving force for the passive diffusion of chloride across the paracellular pathway.

In the early proximal tubule, the transcellular reabsorption of sodium with glucose and amino acids results in a lumen negative potential difference. This lumen negative potential provides a driving force for either chloride absorption or sodium secretion across the paracellular pathway. Whether chloride is absorbed or sodium is secreted is dependent on the relative permeabilities of chloride to sodium in the proximal tubule. The relative chloride to sodium permeability is higher in the adult than the neonate, so passive chloride transport secondary to the lumen negative potential difference in the early proximal tubule does not occur to a significant degree in the neonate [48, 248]. The luminal chloride gradient in the late proximal tubule results in a driving force for chloride diffusion across the paracellular pathway [48, 248]. The diffusion of

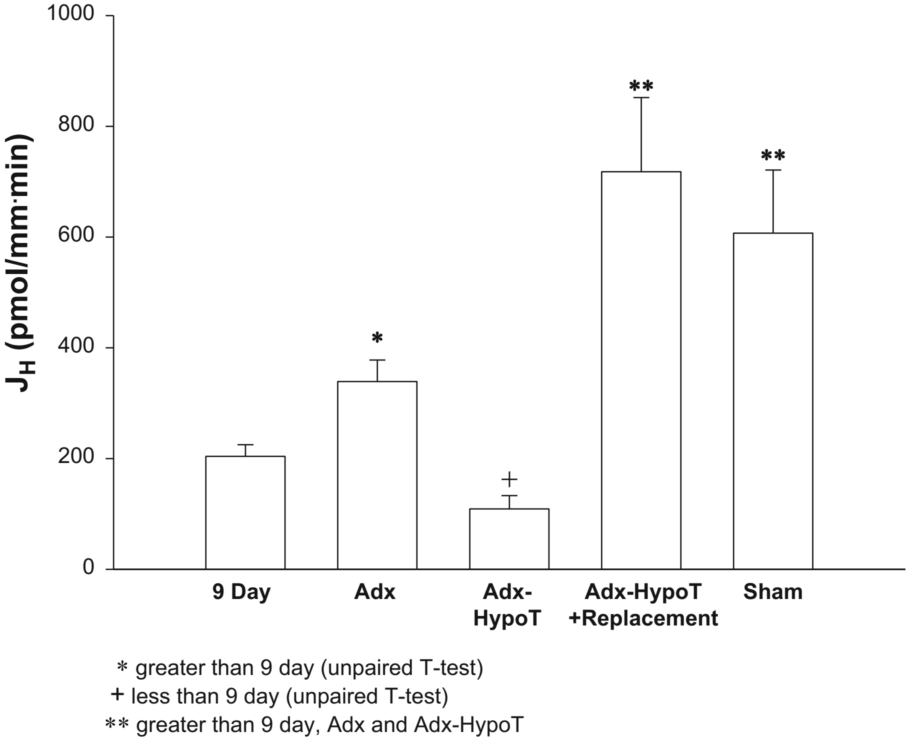


Fig. 11 Apical Na$^+$/H$^+$ exchanger activity in perfused rat tubules. These include 9-day-old rats, and adults that were adrenalectomized as neonates (Adx), adults that were adrenalectomized and hypothyroid (HypoT) since the neonatal period, adults that were adrenalectomized and hypothyroid since the neonatal period but given thyroid and glucocorticoid replacement before study and sham controls. The 9-day-old rats had a lower rate of Na$^+$/H$^+$ exchanger activity than the sham adult. Neonatal adrenalectomy did not totally prevent the maturational increase in Na$^+$/H$^+$ exchanger activity, but the maturational increase in Na$^+$/H$^+$ exchanger activity was abrogated in the adrenalectomized hypothyroid group. (From Ref. [245], with permission)

chloride across the paracellular pathway results in a lumen positive potential difference, and a driving force for the paracellular transport of sodium.

In the adult proximal tubule, approximately half of sodium chloride transport is active and transcellular, and half is passive and paracellular [244, 249, 250]. Active chloride transport is mediated by parallel action of the Na$^+$/H$^+$ and Cl$^-$/base exchangers on the apical membrane [244, 251–253]. It is still somewhat unclear which base is exchanged for chloride as there is evidence for chloride exchange for hydroxyl, formate, and oxalate ions [244, 254–258]. The rate of active transcellular chloride transport is lower in the neonate than that in adults. This is due to the lower rate of the apical Cl$^-$/base exchanger [244, 259] and the Na$^+$/H$^+$ exchanger discussed above [213, 218, 219]. The driving force for active transcellular NaCl transport is the basolateral Na$^+$,K$^+$-ATPase that has lower activity in the neonatal proximal tubule [73, 74, 260]. There are a number of factors that regulate proximal tubule NaCl transport including renal nerves, dopamine, and angiotensin II. The serum levels of most hormones that regulate sodium absorption in the neonate are equal to or higher than in the adult, although in general neonates have a blunted response to most regulatory hormones.

There are also changes in the properties of the paracellular pathway during postnatal development [48, 248, 261, 262]. Most importantly, the permeability of the proximal tubule to chloride ions is lower in the neonate than the adult [48, 248, 262]. The low permeability to chloride ions results in almost no passive paracellular chloride transport in the neonate [48, 248]. As discussed, the permeability properties of an epithelium are determined by the expression of a family of proteins called claudins. The claudin proteins in the tight junction change during the postnatal development. Claudins 6, 9, and 13 are present in the neonatal proximal tubule but not in the adult [261]. Claudin 6 and 9 decrease the chloride permeability of the proximal tubule and increase the paracellular resistance [263].

Of the potential secreted factors that cause the maturational changes in paracellular chloride transport, only thyroid hormone has been examined [264]. Administration of thyroid hormone prior to the normal maturational increase results in an increase in chloride permeability [264]. In contrast, maintaining a hypothyroid state into adulthood prevents the maturational increase in chloride permeability. It is as yet to be determined if thyroid hormone is the endogenous factor that causes the proximal tubule claudin isoform changes during postnatal development.

Proximal Tubule Water Transport

The proximal tubule reabsorbs most of the glomerular filtrate without a significant change in the luminal osmolality. For this to occur, the proximal tubule must be very permeable to water. Proximal tubule water movement occurs both across the cell and paracellular pathway [265–267]. The constitutively water permeable proximal tubule and thin descending limb have water channels on the apical and basolateral membranes [36]. The isoform of this water channel present in the proximal tubule is aquaporin 1 [268–271]. Direct evidence of transcellular water transport comes from aquaporin 1 knockout mice, which have a marked decrease in proximal tubule sodium and water absorption [265, 272]. There is a paucity of water channels in the fetal kidney and an increase in expression of aquaporin 1 does not occur until birth [273, 274]. Deletion of claudin 2, a protein in the tight junction of the proximal tubule decreases proximal tubule transport by 25%, consistent with some water reabsorption via the paracellular pathway [265].

Direct measurements of water permeability have demonstrated that the neonatal rabbit proximal tubule has a higher water permeability that that of the adult [275]. To determine the mechanism for the higher water permeability in neonatal rabbit tubules, studies were performed examining the water permeability of apical and basolateral membrane vesicles [276–278]. Despite the higher transepithelial water permeability, the water permeability of the apical and basolateral membrane were lower in the neonate than the adult and as expected there was less aquaporin 1 expression on both the apical and basolateral membranes of the proximal tubule of the neonate [277, 278]. The apparent paradox between the higher water permeability in the neonatal proximal tubule and the lower water permeability of the apical and basolateral membranes was resolved with measurements of the contribution of the intracellular compartment to water movement in the neonatal and adult proximal tubule. The intracellular compartment was found to cause a large resistance to water flow in adult tubules, but neonatal proximal tubule intracellular compartment was less of a constraint to transcellular water movement [266].

The postnatal increase in glucocorticoids levels is a likely factor in mediating the above maturational changes in water permeability and aquaporin 1 expression. Administration of glucocorticoids to neonatal rabbits resulted in an increase in brush border membrane water permeability and aquaporin 1 expression [279]. Conversely, the developmental increase in thyroid hormone was shown not to affect postnatal maturational changes in water transport [280].

Thick Ascending Limb

The thick ascending limb is responsible for the reabsorption of 30% of filtered NaCl. Mutations in the transporters on the apical and basolateral membrane responsible for transport in this segment result in Bartter Syndrome (see ▶ Chap. 41, “Inherited Disorders of Sodium and Potassium Handling”). The thick ascending limb and the distal convoluted tubule are impermeable to water and the reabsorption of NaCl without water generates a luminal fluid with an osmolality of ~50 mOsm,Kg water. In the absence of ADH action on the collecting tubule, this dilute urine will be excreted. The medullary interstitial hypertonicity is largely generated by active NaCl reabsorption without water by the medullary thick ascending limb.

Reabsorption of NaCl is due to secondary active transport with the basolateral Na^+,K^+-ATPase generating a low intracellular sodium to provide a driving force for luminal sodium entry (see Fig. 12). Sodium enters the cell vial the loop diuretic sensitive $Na^+/K^+/2Cl^-$ cotransporter [281, 282]. The $Na^+/K^+/2Cl^-$ cotransporter is electroneutral yet there is a lumen positive transepithelial potential difference in the thick ascending limb [283]. The positive luminal potential is generated by apical potassium recycling via the potassium channel, ROMK1 [284, 285]. With recycling of potassium back into the lumen, there is the net reabsorption of one sodium and two chloride ions which exit the cell across the basolateral membrane. Sodium exits the cell via the basolateral Na^+,K^+-ATPase. Chloride predominantly exits the cell via a chloride channel designated ClC-Kb [286–288]. A subunit of this chloride channel designated barttin is important in the function of ClC-Kb [289]. The lumen positive potential difference, generated by potassium secretion into the lumen, generates a driving force for the passive paracellular reabsorption of cations including Mg^{++} and Ca^{++} [290–294]. The unique permeability properties of the thick ascending limb are due to the expression of claudin 16 and claudin 19 which interact synergistically to increase cation permeability across the paracellular pathway [295]. Elimination of either claudin 16 or claudin 19 affects the expression of the other protein in the tight junction and decreases cation permeability [295, 296]. Claudin 16 or claudin 19 are mutated in familial hypomagnesemia with hypercalciuria and nephrocalcinosis where there is renal magnesium and calcium wasting [295, 297]. Claudin 19 is also present in the eye and patients with claudin 19 mutations also have vision problems [298, 299].

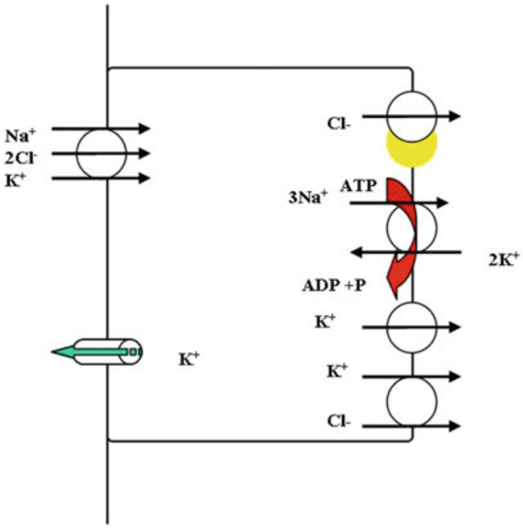


Fig. 12 Diagram of a thick ascending limb cell. On the apical membrane is the $Na^+,K^+/2Cl^-$ cotransporter that is sensitive to loop diuretics and the potassium channel designated ROMK (renal outer medullary potassium channel) that is depicted as the green arrow. The basolateral membrane has a Na^+,K^+-ATPase, a KCl cotransporter, a chloride channel designated ClC-Kb, with its accessory channel designated barttin in yellow and a potassium channel. A loss of function mutation in the $Na^+K^+/2Cl^-$ cotransporter, ROMK, ClC-Kb, or barttin results in Bartter's syndrome

Mutations of the $Na^+/K^+/2Cl^-$ cotransporter [300], ROMK [301, 302], ClC-Kb [303], and barttin [289, 304] all cause Bartter syndrome. Barttin is also expressed in the ear where it is a subunit of ClC-Ka and ClC-Kb. Mutations in barttin cause sensory neural hearing loss [289, 304]. A fifth type of Bartter syndrome is due to an activating mutation of the calcium-sensing receptor [305, 306]. Patients with this latter autosomal dominant disorder have hypocalcemia due to activation of the calcium-sensing receptor in the parathyroid glands. In addition, the calcium-sensing receptor is expressed on the basolateral membrane of the thick ascending limb and activation of the receptor results in inhibition of both $Na^+/K^+/2Cl^-$ cotransporter and ROMK [305].

Micropuncture studies examining fluid from the early distal tubule found that the osmolality of the fluid was significantly lower in the adult than the neonatal rat [307]. This is consistent with lower rates of sodium transport in the neonatal thick ascending limb, although this study did not examine the latter parts of the diluting segment. Sodium transport in the thick ascending limb has been directly examined in vitro and shown to be fivefold lower in the neonate than in the adult [308]. Human neonates are able to dilute their urine to a comparable level as an adult (~50 mOsm,Kg water). This is vital since neonates ingest mother's milk, which is a hypotonic fluid. The ability of a neonate to dilute urine to the same extent in spite of a lower rate of sodium transport as an adult is due a lower glomerular filtration rate and delivery of sodium chloride to this segment in the neonate.

The $Na^+/K^+/2Cl^-$ cotransporter can first be detected in the mid-late gestation rat thick ascending limb and macula densa [309]. Rats have a postnatal maturational increase in $Na^+/K^+/2Cl^-$ cotransporter, ROMK, Na^+,K^+-ATPase mRNA and protein abundance but no change in ClC-K mRNA abundance [310]. Administration of dexamethasone before the normal maturational increase at the time of weaning resulted in a premature increase in urinary concentrating ability and increase in $Na^+,K^+/2Cl^-$ cotransporter, Na^+,K^+-ATPase mRNA and protein abundance, but no change in ROMK protein abundance [310]. There is also a postnatal maturational increase in thick ascending limb Na^+,K^+-ATPase activity [74, 311]. The maturational increase in thick ascending limb Na^+,K^+-ATPase activity is accelerated precociously by glucocorticoids and prevented by neonatal adrenalectomy [311, 312].

Distal Convoluted Tubule

The distal convoluted tubule reabsorbs approximately 5% of the filtered sodium. This segment is water impermeable and further reabsorption of salt without water occurs in this segment resulting in the nadir of luminal fluid osmolality. The transporters responsible for NaCl transport in the distal convoluted tubule are shown in Fig. 13. Sodium enters the cell via the thiazide-sensitive cotransporter [313–316]. Mutations in the thiazide-sensitive cotransporter cause Gitelman's syndrome [317–319] (see ▶ Chap. 41, "Inherited Disorders of Sodium and Potassium Handling"). There is also evidence for parallel Na^+/H^+ and Cl^-/base exchange mediating sodium entry in the rat distal convoluted tubule [320]. NHE2 is the isoform of this Na^+/H^+ exchanger [321]. Chloride exits the cell via a basolateral chloride channel ClC-K [322] and a KCl cotransporter KCNQ1 [323, 324]. There is also a basolateral potassium channel [325]. While active transcellular calcium and magnesium transport occurs in the distal convoluted tubule, there is no information about the postnatal developmental expression of these transporters [326, 327].

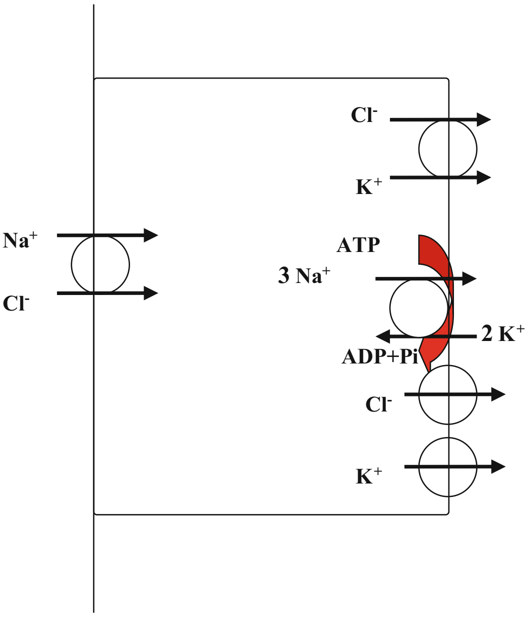


Fig. 13 Distal convoluted cell showing the transporters involved in active sodium reabsorption. The apical NaCl transporter is the thiazide-sensitive cotransporter. Inactivating mutations in the thiazide-sensitive cotransporter lead to Gitelman's syndrome

The distal convoluted tubule is very difficult to study in vitro and in vivo. No studies have been performed to date examining the relative abundance of the thiazide-sensitive cotransporter in the neonate and adult. One study suggests that there is increased sodium absorption in 24-day-old compared to 40-day-old rats [328]. That said, many studies have shown that the neonate is less able to excrete a sodium load compared to the adult [328–330]. For example, administration of isotonic saline equal to 10% of an adult dog's weight resulted in excretion of 50% of the salt load over 8 h, while a similar infusion in a 1–2-week-old dog led to only 10% of the salt load being excreted [328]. This limited ability to excrete a sodium load in the neonatal dog was not due to the lower glomerular filtration rate [328]. Since neonatal sodium transport is reduced in the proximal tubule, thick ascending limb, and collecting tubule, the nephron segment where there was avid neonatal sodium reabsorption was unclear. It is possible that the volume of distribution of the saline infusion was greater in the neonate than the adult, which may account for these findings.

A micropuncture study suggested that the sodium retention in the neonate was the result of avid sodium reabsorption in the distal convoluted tubule [331]. The tubular fluid to plasma Na/inulin (a volume marker) was greater in the early distal tubule in the 24-day-old neonatal rat compared to a 40-day-old rat due to decreased proximal and loop sodium absorption in the younger rat. This difference disappeared in the late distal tubule indicating that the 24-day-old rat had a higher rate of sodium absorption in that distal convoluted tubule. While volume expansion resulted in a higher distal delivery of sodium from the early proximal tubule of the 24-day-old rat, there was less sodium remaining by the end of the distal nephron; this is consistent with the distal convoluted tubule being the segment responsible for decreased ability of neonates failure to excrete a sodium load versus adults.

Urinary Concentration and Dilution

The urine exiting the distal convoluted tubule and entering the collecting duct has an osmolality of ~50 mOsm/Kg water. Whether the urine will be of this osmolality or maximally concentrated will depend on the presence or absence of vasopressin (ADH). Neonates can dilute their urine to nearly the adult level of nearly 50 mOsm/Kg water [332–334]. Thus the neonate which imbibes mother's milk must excrete high amounts of free water [333–335]. However, unlike the normal adult, where it is almost impossible to drink enough water to cause hyponatremia (30 l/day), neonates have a rather limited ability to generate and excrete free water and improperly mixed dilute formula can result in hyponatremia [334].

The maximum urine osmolality of the human neonate is ~400–600 mOsm/Kg water [336–338]. The adult urine osmolality can be achieved at 1.5–2 years of age [337]. There are many potential developmental factors that could limit the ability of the neonate to generate maximally concentrated urine. The factors involved in urine concentration include:

1. The ability to sense an increase in serum osmolality or decrease in extracellular volume and secrete vasopressin
2. Presence of vasopressin receptors on the basolateral membrane of the collecting duct
3. Ability of the collecting duct to generate cAMP
4. Ability of the loop of Henle to generate a concentrated interstitium
5. Presence of medullary architecture to allow maximal concentrating ability
6. Presence of aquaporins on the apical and basolateral membrane of collecting tubules for the transcellar passage of water

During prenatal and postnatal maturation, there are anatomical changes which occur that likely affect urinary concentrating ability. There is an increase in the medullary capillary density, a decrease in medullary interstitial connective tissue, and an increased presence and length of the thin limbs; also the tubules become more tightly packed with cells in the loop decreasing in height with maturation [339, 340]. The length of the papilla increases linearly from day 10 to day 40 in the rat [341]. All these developmental anatomical changes are necessary for the countercurrent multiplication system to be maximally efficient. Accompanied by these anatomical changes are concomitant increases in the medullary sodium and urea concentration [341, 342]. Administration of a high protein diet or urea to human neonates increases the neonates concentrating ability [343, 344]. Thus, part of the limited concentrating ability is the result of the protein content of the diet. Adrenalectomy in neonatal rats prevented the maturational increase in urine osmolality while administration of glucocorticoids prior to the maturational increase in serum glucocorticoid levels results in a premature increase in urinary concentrating ability [341].

Principal cells in the collecting tubule and the cells in the medullary collecting duct express aquaporin 2 on the apical membrane and aquaporins 3 and 4 on the basolateral membrane [345–349]. Aquaporin 3 null mice have diabetes insipidus whereas aquaporin 4 null mice only have a small concentrating defect after water deprivation indicating that basolateral water movement is primarily via aquaporin 3 [350, 351]. Vasopressin causes the intracellular vesicles containing aquaporin 2 to fuse with the apical membrane resulting in the insertion of aquaporin 2 into the apical membrane [346]. There is a developmental increase in aquaporin 2 expression [347, 348, 352]. The maturational increase in aquaporin 2 is accelerated by administration of synthetic glucocorticoids prior to the normal postnatal increase in plasma glucocorticoids [352]. Neither aquaporin 3 nor aquaporin 4 impair urinary concentration in the neonate [347, 353].

The fetus and neonate respond to increases in serum osmolality, stress, and hypovolemia with appropriate increases in plasma vasopressin levels. The fetal sheep has an increase in plasma vasopressin with an infusion of hypertonic saline and increase in plasma osmolality [354–356]. Plasma vasopressin levels also increase in fetal sheep in response to volume depletion induced by hemorrhage or diuretics [356–358] Despite the increases in fetal sheep serum vasopressin levels with stress, infusion of vasopressin in non-stressed fetal sheep resulted in a blunted increase in urine osmolality compared to adult sheep [359]. While it is hard to assess these issues in humans, comparison between a relatively stressful vaginal delivery to a cesarean section has consistently demonstrated higher vasopressin levels in neonates born vaginally [360–363]. There was no correlation with vasopressin levels and the degree of perinatal asphyxia in one study [360], while there was a correlation in another [362]. In sum, it appears that the fetus and neonate can respond to appropriate stimuli with vasopressin secretion, but may have a blunted response to vasopressin.

There is a developmental increase in vasopressin receptors in the kidney; however, vasopressin receptor abundance does not appear to be a limiting factor in urinary concentration in the neonate [364]. Exogenous administration of vasopressin increases the urine osmolality in fetal sheep showing that vasopressin action on the collecting tubule is a prenatal event [359]. Vasopressin acts in the collecting tubule by increasing cAMP. There is some discrepancy about the extent of cAMP production during postnatal maturation in comparison to adults. The sum of the data support that while vasopressin stimulates cAMP production in the neonate, the amount of cAMP generated is attenuated compared to the adult [364–367].

The response of the collecting tubule to vasopressin has been examined in vitro [368–370]. The increase in water permeability in neonatal collecting ducts in response to vasopressin is less than in the adult tubule [368–371]. Studies have shown that the phosphodiesterase activity is greater in the neonatal collecting tubule than the adult [370]. Inhibition of phosphodiesterase activity in neonatal collecting tubules results in comparable water permeability to that of adult tubules demonstrating that enhanced degradation of cAMP in the neonatal collecting tubule likely limits the vasopressin induced increase in water permeability [370]. Vasopressin also increases prostaglandin production in the collecting tubule which in turn attenuates the vasopressin mediated increase in cAMP production [372, 373]. Vasopressin-mediated cAMP production, while less in the neonatal collecting tubule, increases to adult levels in the presence of indomethacin, a prostaglandin synthesis inhibitor [372]. Thus, prostaglandin production by the collecting tubule attenuates the effect of vasopressin in the neonatal tubule to a greater extent than the adult tubule [372].

Distal Tubule Acidification

The distal nephron makes the final adjustment in distal acidification. The distal nephron, under normal circumstances secretes the protons equal to that generated from metabolism and in growing children, the protons liberated from the formation of bone as well. The cortical collecting tubule has two types of cells that are involved in renal acidification. The α-intercalated cell is responsible for proton secretion and is shown in Fig. 14. There is also a β-intercalated cell (see Fig. 14), which can secrete bicarbonate when the animal is alkalotic or eats a diet with a high alkali content [374]. This is atypical for most humans, but not for some mammals which can ingest an alkali diet [375]. The β-intercalated cell has the reverse polarity as the α-intercalated cell but the Cl^-/HCO_3^- exchanger on the apical membrane of the β-intercalated cell is different than that on the apical membrane of the α-intercalated cell.

The neonatal cortical collecting tubule has fewer and less differentiated intercalated cells than that of the adult segment [376, 377]. The rates of both bicarbonate secretion and luminal

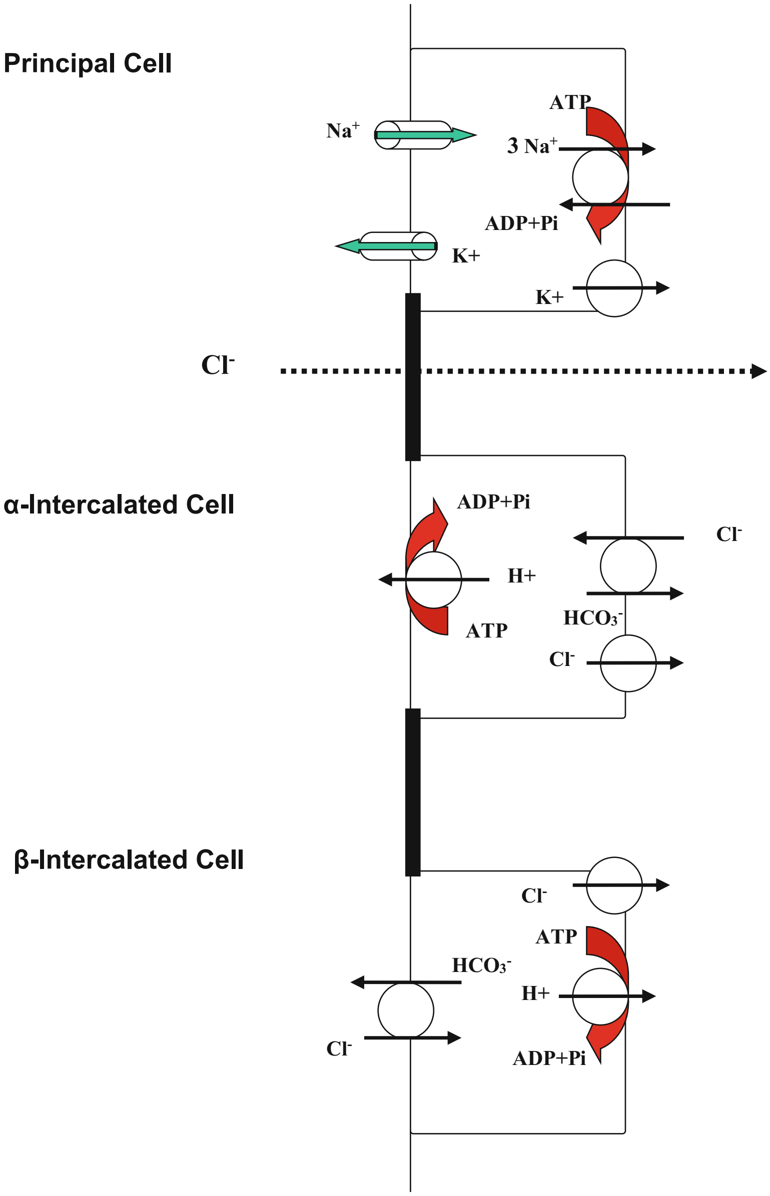


Fig. 14 Three cells of the cortical collecting tubule are demonstrated. The principal cell (top) is shown with sodium entering the cell down its electrochemical gradient via a channel designated ENaC. This generates a lumen negative transepithelial potential difference. Potassium is secreted into the lumen down its electrochemical gradient via ROMK. Chloride moves through the paracellular pathway down the electrical gradient generated by the lumen negative potential difference. The alpha-intercalated cell (middle) secretes protons into the cell lumen via a H^+-ATPase. The bicarbonate generated in this process is secreted across the basolateral membrane via a Cl^-/HCO_3^- exchanger. The beta intercalated cell (bottom) secretes bicarbonate ions in the face of metabolic alkalosis. This cell has the reverse polarity of an alpha-intercalated cell but the Cl^-/HCO_3^- exchanger is different isoform

acidification are reduced in the neonate than in the adult [375, 377, 378]. The intercalated cells in the neonatal inner medullary collecting duct are much more differentiated in appearance than in earlier tubular segments and secrete protons at a rate comparable to the adult [375, 378]. The collecting tubule also has a luminal H^+-K^+ ATPase that secretes protons and reabsorbs potassium from the luminal fluid. It is activated under states of metabolic acidosis and hypokalemia [379]. The H^+-K^+ ATPase can function at comparable rates in the neonatal cortical collecting tubule as that of the adult [380].

Cortical Collecting Tubule Sodium Transport

While relatively little sodium is reabsorbed by the cortical collecting tubule compared to the more proximal nephron segments, it is the final nephron segment responsible for regulating sodium absorption and thus is vitally important for the regulation of sodium homeostasis. Sodium absorption occurs in the principal cell of the collecting tubule, which is shown in Fig. 14. Sodium transport occurs through the epithelial sodium channel designated ENaC which has three subunits. The driving force for sodium entry across ENaC is the low intracellular sodium concentration and the potential difference across the luminal membrane generated by the basolateral Na^+,K^+-ATPase. The Na^+,K^+-ATPase undergoes a maturational increase in this segment, but is not the limiting factor the decrease in neonatal sodium absorption [74]. Trafficking of ENaC to the apical membrane is regulated by aldosterone and vasopressin [381, 382]. Compared to the adult, aldosterone has a blunted effect in the neonate despite the fact that there are higher serum potassium levels in the neonate than adults, and ample aldosterone receptors in neonates [383–389].

Sodium transport in the cortical collecting tubule increases with postnatal development [388, 390]. This increase in sodium transport is paralleled by an increase in the apical expression of ENaC, which is the limiting factor in sodium absorption in this segment [391]. ENaC is composed of a-, ß-, and γ- subunits, and there are developmental increases in mRNA and protein abundance of each during postnatal maturation [391–395]. However, the increase in mRNA abundance occurs shortly after birth in the rat while the increase in protein abundance occurs latter, consistent with posttranscriptional regulation of the ENaC subunits during postnatal development [393]. The limitation to sodium transport via ENaC is not only due to the limited apical expression, but also due to the fact that the transporters on the neonatal apical membrane have a greater probability of being closed rather than open to let sodium into the cell compared to adult transporters [391].

Potassium Transport

Neonates have higher serum potassium levels than adults. Potassium is the predominant intracellular cation and neonates must be in positive potassium balance for growth unlike adults, which excrete the quantity of potassium absorbed from their diet into their urine [379]. Adult animals are more readily able to excrete an exogenous potassium load than is a neonate [396]. Approximately half of the filtered potassium is reabsorbed in the proximal tubule of the adult and neonate by passive diffusion across the paracellular pathway [397]. The loop of Henle reabsorbs the remaining 80% of the delivered potassium in the adult, but only 45% of the delivered potassium in the neonate [397]. Thus, the adult delivers approximately 10% of the filtered potassium to the distal nephron while the neonate delivers 25%. The distal convoluted tubule, connecting tubule, and cortical collecting duct are the sites of potassium secretion and final modulators of urine potassium excretion.

Potassium secretion by a principal cell is depicted in Fig. 14. Sodium enters the principal cell through the apical sodium channel down the favorable electrochemical gradient generated by the Na^+,K^+-ATPase on the basolateral membrane. This results in a lumen negative potential difference and a favorable electrochemical gradient for potassium secretion. While there is a maturational increase in the sodium channel and the Na^+,K^+-ATPase, these are not the limiting factors for potassium secretion. There are two potassium channels on the apical surface of the principal cells of collecting tubule: a channel designated ROMK, and a channel designated the maxi-K channel (also known as the BK channel) that is a flow-dependent channel and activated by stretch [379]. There is a maturational increase in potassium secretion in cortical collecting tubules which was demonstrated by in vitro tubule perfusion [390]. Secretion of potassium by principal cells is paralleled by the maturational increase in potassium channels (ROMK) on the apical membrane of the principal cell [395, 398]. The number of ROMK channels increases during development, but the open probability remains constant [398]. There is also a developmental increase in the flow regulated maxi-K channels [399]. Potassium secretion is regulated by aldosterone and there is resistance to the action of aldosterone despite higher serum levels in the neonate [383–389].

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3

Congenital Anomalies of the Kidneys

Paul Goodyer, Indra R. Gupta, Rik Westland, and Ihor Yosypiv

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Abstract

Congenital kidney anomalies are the result of embryonic maldevelopment that dates back to the fifth gestational week in humans. At this time-point, the metanephric mesenchyme, which originates from the nephrogenic cord, and the outgrowing ureteric bud, which originates from the nephric duct, display highly coordinated spatiotemporal interactions that result in the development of the kidney and the adjacent urinary tract (see ▶ Chap. 1, "Pre-natal Development of the Kidneys and Urinary Tract"). Disturbances in the molecular communication between the metanephric mesenchyme and the ureteric bud give rise to a spectrum of kidney abnormalities that differ from one another depending on the timing and/or location of the disturbance. Based on the location of the disturbance, congenital kidney anomalies can arise as unilateral or bilateral defects, or they can result in the formation of ectopic kidney tissue. Early perturbations lead to the complete absence of kidney tissue, whereas later events may result in an underdeveloped kidney that is hypoplastic or dysplastic or in a kidney with reduced nephron number. Many of these spatiotemporal disturbances are caused by genetic, epigenetic and/or environmental factors that are also involved in the development of other organs. Therefore, congenital kidney defects may present in conjunction with extrarenal defects or be part of a well-defined clinical syndrome.

In this chapter, we discuss the phenotypic spectrum of human congenital kidney defects that encompasses unilateral renal agenesis (URA), bilateral renal agenesis, renal hypoplasia, renal dysplasia, multicystic dysplastic kidney (MCDK) as well as ectopic kidney tissue, kidney fusion defects (such as crossed fused ectopia and horseshoe kidney), and finally, duplication of the kidney. The clinical presentation, their management, and the syndromes that are associated with congenital kidney anomalies will be discussed. Finally, we provide a state-of-the-art overview of the molecular mechanisms that give rise to congenital kidney anomalies.

Keywords

Metanephric mesenchyme · Ureteric bud · Syndromes · Next generation sequencing · Copy number variants

Synonyms

Renal dysplasia; Renal hypodysplasia; Renal hypoplasia

Clinical Presentation of Congenital Anomalies of the Kidneys

Antenatal Presentation of CAKUT

Using transvaginal ultrasound, urine first appears in the human bladder at the ninth week of gestation. However, the onset of urine flow and its contribution to amniotic fluid volume is not easily detected by transabdominal ultrasonography until 16 weeks or more. Thus, most disturbances of embryonic renal patterning that lead to congenital anomalies of the kidney and urinary tract (CAKUT) are usually identified at the 20–22-week ultrasound, now widely performed as part of routine antenatal care. Key findings range from sequelae of urinary tract obstruction to monogenic disorders that disturb kidney position to disturbances of cellular patterning in renal glomeruli or tubules. Subsequent ultrasound tests or more refined imaging techniques (i.e., magnetic resonance imaging) later in pregnancy allow physicians to track the evolution of these renal anomalies.

Renal Agenesis

Bilateral renal agenesis (BRA) occurs in about 1: 10,000 live births. Lack of kidney-derived amniotic fluid causes a characteristic pattern of facial compression, limb contractures, amnion nodosum, and pulmonary hypoplasia (Potter syndrome) that limits postnatal viability (Fig. 1). By compromising fetal respiratory movements that optimize fetal lung development, severe oligohydramnios may limit gas exchange and result in an urgent need for intubation and intensive respiratory support after birth.

In most cases, the cause of BRA is not known. However, reports of the absence of renal arteries and ureters in the second trimester suggest arrest of kidney development at an early stage. Roodhooft et al. examined 40 siblings of 41 index cases with BRA and found that 10% had unilateral RA (URA), suggesting a heritable cause [1]. In an Australian cohort, URA was found in siblings of babies with isolated BRA but not in siblings of cases of BRA with multiple malformations [2]. BRA has been associated with recessive mutations in the gene *integrin alpha 8* (*ITGA8*) that encodes a transmembrane receptor important for cell adhesion and cell signaling between the metanephric mesenchyme and ureteric bud [3]. BRA has also been associated with recessive mutations in *fibroblast growth factor 20* (*FGF20*) that is expressed by nephron progenitor cells during kidney development [4], X-linked Kallmann syndrome due to mutations in the *KAL-1* gene, an extracellular matrix protein expressed in the kidney [5], and rarely with heterozygous mutations in the gene encoding the receptor tyrosine kinase *RET* that is expressed in the ureteric bud [6].

Unilateral renal agenesis occurs in about 1:2,000 live births (Fig. 2). The incidence of URA in males (63%) is slightly higher than in females, and the left kidney is more often absent (63%) than the right [7]. Westland et al. reviewed 2,684 patients with URA from many pediatric and adult cohorts and found that about one third had various urinary tract anomalies, including vesicoureteral reflux (24%), ureteropelvic junction obstruction (6%),

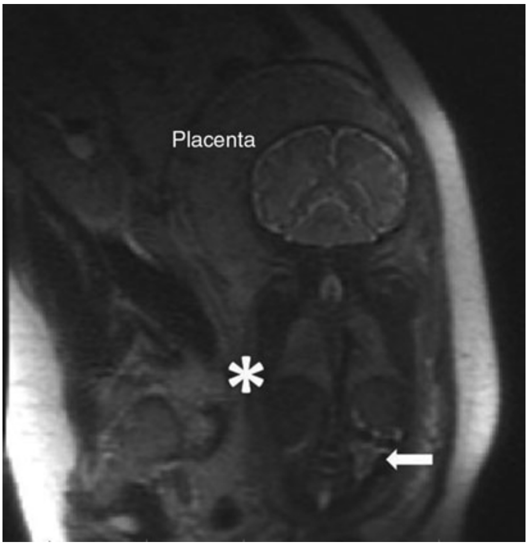


Fig. 1 Magnetic resonance image showing case of Fraser syndrome with the absence of the right kidney, hypoplastic left kidney (*arrow*), and anhydramnios (*asterisk*)

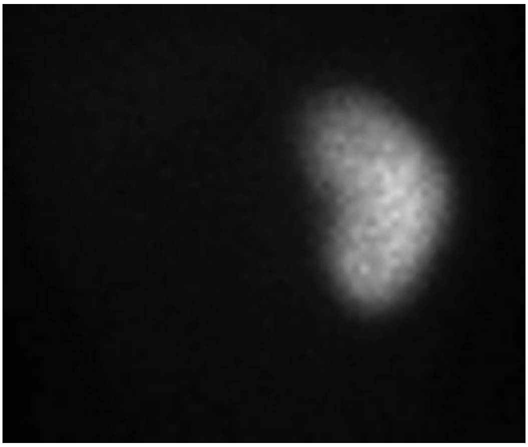


Fig. 2 DMSA scan showing agenesis of the left kidney

megaureter (7%), ureterocoele (1%), and posterior urethral vales (1%) [8]. One third of cases were associated with syndromic extrarenal anomalies. From analysis of a subset of the cohorts, it was determined that the mean GFR was 100 ml/min/ 1.73 m^2 and that the majority of patients with an isolated solitary functioning kidney have stable renal function for many decades. However, 21% had microalbuminuria, 16% had hypertension, and 10% had a glomerular filtration rate <60 ml/min/ 1.73 m^2. It is unclear whether this renal dysfunction is the consequence of a congenital nephron deficit with or without dysplasia in the remaining kidney. In a pediatric cohort of URA/MCDK (multicystic dysplastic kidney) patients from the Netherlands [9], hypertension was seen in 31%, and microalbuminuria was present in 23%, indicating that these issues are generally evident before the age of 18 years.

URA reduces total nephron mass and usually induces compensatory renal hypertrophy in the remaining kidney during fetal life. In an ultrasonographic study of a mixed URA/MCDK cohort, the length of the single functioning kidney was estimated to be 23% longer than normal from the 20th week of gestation onward [10]. Cho et al. proposed that the ratio of the anterior-posterior to transverse diameter of the kidney discriminates appropriately hypertrophied kidneys (1.0) from the ratio in babies with two normal kidneys (0.8) [11].

Unilateral multicystic, dysplastic kidneys may mimic URA when they have involuted prior to evaluation. The MCDK is actually a form of severe renal dysplasia in which the kidney is occupied by tense non-communicating macrocysts [12]; typically, there is little or no functional parenchyma (Fig. 3a, b, c). The renal pelvis and calyces cannot be identified, and the ureteropelvic junction and ureter are usually atretic; ipsilateral vesicoureteral reflux is rare. The incidence of MCDK ranges from 1 per 2,200 to 4,300 births; it is slightly more common among males (55%) and slightly more common on the right (53%) than on the left [13]. Among males, 5% have ipsilateral or bilateral undescended testes [14]. With widely available antenatal ultrasound screening, MCDK is now increasingly diagnosed before birth but follow-up postnatal ultrasonography is needed to distinguish MCDK from high-grade urinary tract obstruction from ureteropelvic junction obstruction.

Since MCDK is typically unilateral, the long-term renal prognosis depends primarily on the integrity of the contralateral kidney. After long-term follow-up, 2/40 Italian and 2/34 Indian MCDK patients eventually developed end-stage renal failure [15, 16]. In most cases, the estimated glomerular filtration rate is normal, ranging from 86 to 122 ml/min/1.73 m^2 during childhood [17]. About one quarter of the contralateral kidneys have low grade vesicoureteral reflux, grade I–III; therefore, routine cystourethrogram is not usually indicated unless the patient has recurrent urinary tract infections. Less often, the contralateral kidney has ureteropelvic junction obstruction (3–5%) or ureterovesical junction obstruction (2%). In several large series, there were a small number of contralateral kidneys (2–5%) which exhibited severe reflux, remained small and hyperechoic and failed to show normal compensatory hypertrophy. Children with these findings in the contralateral kidney exhibit mild proteinuria (5–30%) and have the highest risk of progressing to end-stage renal failure [18]. Since the MCDK is usually non-functional, this mild proteinuria presumably arises from the contralateral kidney. Hypertension occurs in a small fraction (1–6%) of cases [19], and is usually associated with minimal residual functional parenchyma (noted by renal scan) in the MCDK. Importantly, hypertension is often reported to resolve when the MCDK is resected.

The etiology of the MCDK is unclear but presumably involves a disturbance when the primordial nephrons become linked to the collecting duct system. Importantly, increased gene copy number variation is seen in >10% of MCDK patients [20]. MCDK has also been associated with heterozygous mutations of the *HNF1β* gene (see below) [21].

Renal hypoplasia is another congenital anomaly of the kidneys and typically refers to kidneys that are reduced in size (2 standard deviations below the expected mean when correlated with age or body height). Hypoplastic kidneys usually

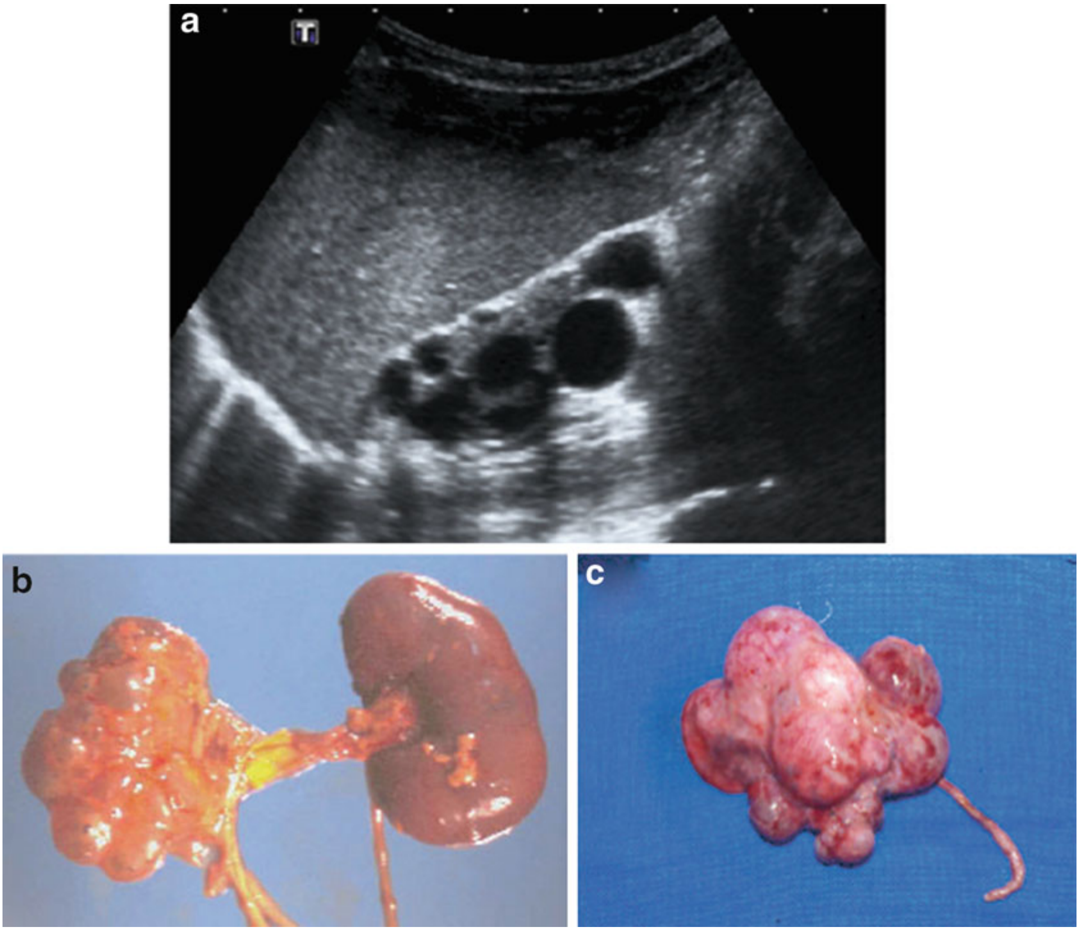


Fig. 3 Multicystic dysplastic kidney. (**a**) Multicystic dysplastic on renal ultrasound imaging (**b**) Multicystic dysplastic kidney (*left*) and normal kidney (*right*) en bloc; (**c**) Multicystic dysplastic kidney in situ

exhibit normal morphology but have reduced nephron numbers. Malformed kidneys may also show features of tissue maldifferentiation with or without cystic change and this is defined as renal dysplasia (Figs. 4, 5, and 6). In fact, renal hypoplasia and dysplasia can only be distinguished based on histopathological assessment of renal tissue, which is rarely performed because the distinction is mostly of academic interest. Both pathologies are risk factors for end stage renal disease, especially if there is bilateral renal involvement. The diagnosis of renal hypoplasia or dysplasia is most commonly made based on the findings on ultrasound imaging: the renal hypoplastic kidney will be small in length, while the dysplastic kidney will be hyperechoic, may be small and may exhibit cystic features. A nuclear medicine scan may also support the diagnosis of renal dysplasia (complete or regional) if it reveals a diffuse loss of isotope uptake or if it reveals specific regions where there is no uptake.

Renal Hyperechogenicity and Parenchymal Cysts

At the 20–22-week gestational ultrasound, radiologists may report hyperechogenicity in one or both kidneys, obscuring normal cortico-medullary differentiation. This often represents renal dysplasia associated with microcystic renal disease, since sub-millimetric parenchymal cysts reflect the ultrasound waves at each fluid/solid surface. However, occasional macrocysts (>1 cm diameter) may also be present. Several clinical features can help distinguish the various forms of cystic renal disease that can be identified by antenatal ultrasound [22]. Because of the substantial phenotypic overlap between these genetically heterogeneous subtypes, diagnostic accuracy may be improved by performing a genetic screen for copy number variants and a broad multiplex gene panel (up to 100 genes) to screen for mutations in genes previously associated with renal cysts and/or dysplasia.

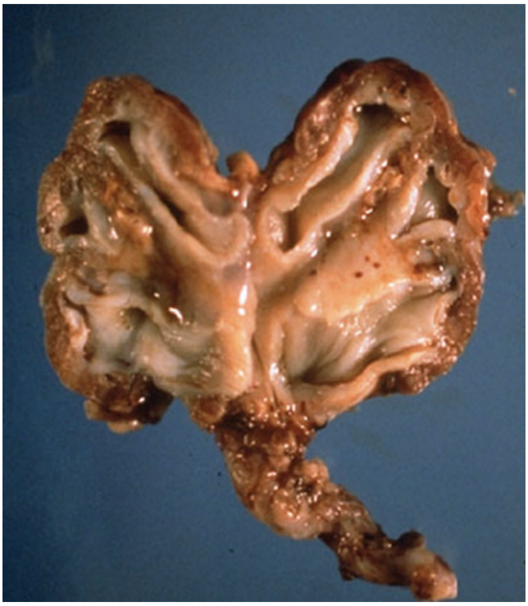
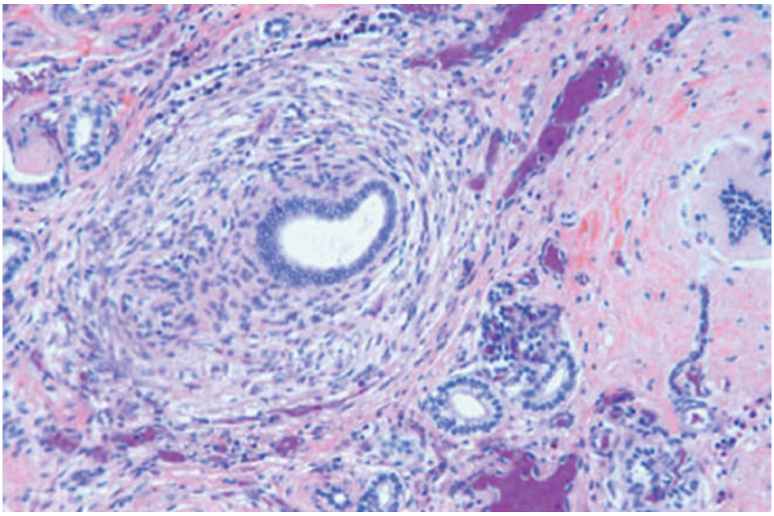


Fig. 4 Image of hypoplastic/dysplastic kidney with bisected pelvis

ARPKD (biallelic mutations of the *PHKD1* gene) represents the classic example of antenatal heterogeneous, hyperechoic kidneys (see ▶ Chap. 50, "Autosomal Recessive Polycystic Kidney Disease"). Both kidneys are typically massively enlarged (3–4 standard deviations above the mean) due to diffuse dilatation of distal collecting tubules (<2 mm diameter) but occasional macrocysts >1 cm may also be seen. Severe ARPKD is often evident by 16 weeks gestation, but the presentation is variable. At the 20–22-week screening ultrasound, amniotic fluid may be normal or reduced; serial ultrasounds are crucial to track progressive reduction in amniotic fluid index (AFI) and lung development, since these issues will influence viability in the perinatal period.

ADPKD (germline heterozygous mutations of the *PKD1* or *PKD2* genes) was previously considered to be a cystic kidney disease of adults, although it is now clear that cystogenesis begins in utero and a subset of patients develop macrocysts in late gestation (3%) or in the early pediatric years (up to 23%) (see ▶ Chap. 49, "Autosomal Dominant Polycystic Kidney Disease"). Antenatal ultrasound may show kidney enlargement, often two standard deviations above the mean in 50%, although the size increase is not usually as extreme as in ARPKD and amniotic fluid is

Fig. 5 Histological image showing peritubular fibrous cuff in an area of renal dysplasia



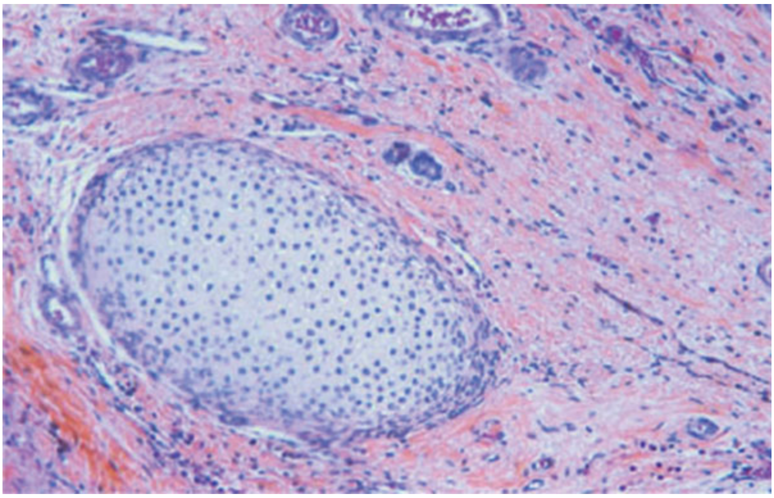


Fig. 6 Histological image showing ectopic cartilage in an area of renal dysplasia

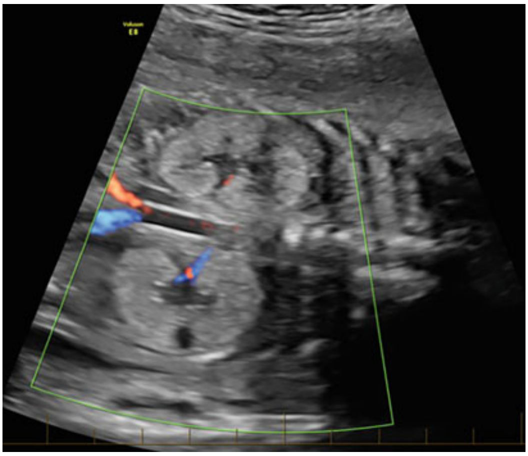


Fig. 7 Ultrasound image of a fetus with hyperechoic kidneys with cysts due to a mutation in HNF1b

typically normal [23]. Multiple antenatal macrocysts (>1 cm diameter) are more commonly seen than in ARPKD. Interestingly, antenatal ADPKD may occasionally mimic ARPKD, especially when the affected fetus harbors biallelic mutations in *PKD1* [24].

Mutations of the *HNF1β* gene are the most common cause of antenatal hyperechoic kidneys on ultrasound imaging [25] (Fig. 7). Nephromegaly and "bright" renal parenchyma may be evident as early as 13 weeks; occasional macrocysts (>1 cm) may appear later in gestation. Amniotic fluid volume usually remains normal in utero and progressive renal insufficiency is unusual before 20 years of age. The highly variable renal phenotype, involving hyperuricemia and diabetes mellitus in young adults, is discussed later in the chapter (see also ▶ Chap. 53, "Autosomal Dominant Tubulointerstitial Kidney Disease").

Renal Ectopia and Fusion Anomalies

During embryogenesis, the two kidneys ascend from a low pelvic position to their normal upper intra-abdominal location. Disturbance of renal ascent leads to renal ectopia in about 1:900 births and is usually (90%) unilateral. The associated ureter nearly always enters the bladder normally, but about half are associated with hydronephrosis. This may be due to ureteropelvic junction obstruction, ureterovesical junction obstruction, or vesicoureteral reflux [26]. In about 40% of cases, the ectopic kidney is found in the pelvis. While most ectopic kidneys are asymptomatic, ectopic kidneys in the lumbar region are sometimes associated with poorly localized pain. Rarely, ectopic kidneys have been reported in the thorax and are identified as an incidental non-functional chest mass.

When aberrant ascent brings them into contact, the two kidneys may fuse. Most commonly, this occurs in the midline and is referred to as a horseshoe kidney (Fig. 8). In 95% of cases, the horseshoe kidney is low-lying (L3-L4) because further ascent has been blocked by the inferior mesenteric artery. The two moieties are fused via a

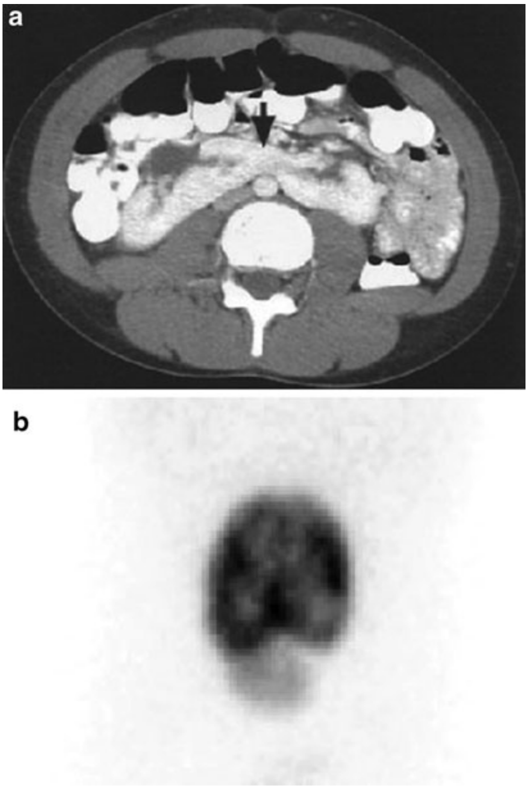


Fig. 8 Horseshoe kidneys: (**a**) Computerized tomography scan showing isthmus of horseshoe kidney fused in midline, (**b**) DMSA scan showing pancake kidney fused in the midline

parenchymal isthmus bridging their lower poles and may develop a variety of vascular arrangements, including a single artery [27]. About half of horseshoe kidneys exhibit vesicoureteral reflux and this can be associated with recurrent urinary tract infections [28]. Patients with horseshoe kidneys are more likely to develop kidney stones at some point during their life. There are occasionally VACTERL (3%) and reproductive tract (5%) anomalies seen in patients with horseshoe kidneys [29]. Although there are rare case reports of renal cell carcinoma arising within horseshoe kidneys, there is no evidence that this occurs more frequently than in the general population. In contrast, patients with horseshoe kidneys do have an increased risk of Wilms' tumor [30]. Between 15% and 30% of girls with Turner syndrome have horseshoe kidneys [31].

Crossed ectopia occurs when one kidney crosses the midline in relationship to the side where its ureter enters the bladder; in 90% of cases, the upper pole of the ectopic kidney is fused to the lower pole of the orthotopic kidney but sometimes this occurs without fusion, and the two kidneys may even exchange sides. Abnormalities of the reproductive tracts are seen in about half of the cases; these include cryptorchidism or absence of the vas deferens in males and vaginal atresia or unilateral uterine abnormalities in females [32].

Diagnosis and Imaging of Congenital Anomalies of the Kidneys

The presence of congenital anomalies of the kidneys is usually suspected from antenatal ultrasound imaging. Standardized antenatal ultrasound reports include several key renal data:

Amniotic Fluid Index (AFI) and Maximal Vertical Pocket (MFP)

During the first 20 weeks of gestation, the amniotic fluid is primarily made up of lung secretions and hydrostatic and osmotic transport of maternal plasma across fetal membranes into the amniotic cavity. By 16 weeks, fetal urine has become the dominant contributor to amniotic fluid. At the time of the 20–22-week ultrasound, fetal urine contributes about 7–10 ml/kg/h to the total volume, while lung secretions contribute about 100 ml/24 h. In counterbalance to this, fetal swallowing removes amniotic fluid up to a rate of 1 L/24 h. Amniotic fluid resorption is also resorbed directly across the amnion into the fetal circulation (intramembranous absorption). Thus, the entire amniotic fluid volume turns over in a 24-h period and steady-state amniotic fluid volume reflects the balance of these processes. Mean amniotic fluid volume increases from about 400 ml at 20 weeks to about 800 ml (range 400–1800 ml) at 32–34 weeks, and then falls gradually to 600 ml at 40 weeks [33].

Table 1 Amniotic fluid index at various gestational ages. Amniotic fluid index (AFI) is calculated by antenatal ultrasonography as the sum of the largest vertical diameter (cm) of amniotic fluid in each of the four quadrants in the image. Mean (50th percentile) and lower limit (2.5th percentile) for various gestational ages are adapted from Moore et al. [35]

AFI (cm)	50th percentile	2.5th percentile
16	12.1	7.3
20	14.1	8.6
25	14.7	8.9
30	14.5	8.2
35	14.0	7.0
40	12.3	6.3

Although amniotic fluid volume cannot be measured precisely in most clinical settings, it can be estimated by transabdominal ultrasonography after 20 weeks gestation. The images of the uterus are divided into four quadrants and, with the transabdominal ultrasound probe held in the sagittal plane, the longest vertical fluid diameters in each quadrant are summed. AFI peaks at about 14–15 cm at 34–38 weeks gestation and then falls to about 11 cm by 40 weeks [34]. Nomograms for AFI in healthy pregnancies as reported by Moore are summarized in Table 1 [35]. By 34–38 weeks, oligohydramnios = AFI<5 cm, low-normal = AFI 5–8 cm, normal = 5–25 cm and polyhydramnios = AFI>25 cm. Alternatively, amniotic fluid volume can be assessed by reporting the Maximal Vertical Pocket (among the 4 quadrants). Normal MVP ranges from 2–8 cm while oligohydramnios = MVP<2 cm. Some clinicians feel that the AFI may underestimate oligohydramnios; therefore, both measurements are often reported. Although oligohydramnios often represents a clinically significant reduction in functional renal mass, it should be noted that it can also be seen with premature rupture of fetal membranes, fetal growth restriction and fetal hypoxia (thought to cause fetal vasopressin release).

Kidney Size

Ultrasound estimates of renal size are important measures of nephron endowment between 20 and 40 weeks gestation. During the first months of postnatal life, compensatory renal hypertrophy may gradually mask the initial relationship between kidney volume and nephron number. Interpretation of this parameter in the antenatal ultrasound report requires access to normative values at different gestational ages. Table 2 summarizes mean renal length (+/− 2 standard deviations) at various gestational ages [36].

Antenatal Imaging

The second trimester ultrasound has a sensitivity of 73–81% and a specificity of 99% in establishing the diagnosis of CAKUT [37]. The sensitivity of ultrasound imaging is highly dependent on the operator's skills and the biological characteristics of the mother and the fetus. For example, it may be difficult to discern fetal anomalies in obese mothers or in the setting of oligo/anhydramnios. This is important because maternal obesity (BMI$\geq$30 kg/m^2) is a risk factor for CAKUT [38]. Oligohydramnios or anhydramnios results in a fetus that is not mobile and there is poor separation of tissue-to-tissue interfaces because of the lack of amniotic fluid: both factors result in poor assessment of fetal anatomy by ultrasound. The poor resolution of fetal anatomy in the setting of oligo/anhydramnios has led to attempts to improve ultrasound imaging by performing diagnostic amnioinfusion.

Fetal magnetic resonance imaging is an important modality to consider in the setting of oligo/anhydramnios because low amniotic fluid is less problematic for image quality (see also ▶ Chap. 6, "Imaging Kidneys and the Urinary Tract"). The kidneys, ureters and bladder can be correctly identified from week 20 onwards by MRI. High quality images can be obtained independent of maternal body habitus, fetal position, or the volume of amniotic fluid. MRI is especially useful to define complex urogenital pathology, to locate ectopic kidneys, to assess for other organ disease, and to differentiate between the urinary tract and the distal intestine as in megacystis microcolon intestinal hypoperistalsis syndrome [39]. There do

Table 2 Kidney size by gestational age. Sagittal kidney length and renal volumes at various gestational ages; mean (50th percentile) and lower limit of normal (5th percentile) are shown. (Adapted from van Vuuren et al. [36])

Gestational age (weeks)	*Kidney length (mm)*		*Kidney volume (ml)*	
	50th %	5th %	50th %	5th %
15	12.5	10	–	–
20	20	16	2	1.5
25	26	23	4	2.5
30	33	28	7	4
35	38	34	12	7.5
40	44	38	17	11

not appear to be any harmful effects to the fetus from MRI even when exposed to electromagnetic fields up to 1.5 tesla (T), although ongoing long-term follow-up is needed [40]. Gadolinium should be avoided as a contrast agent for fetal MRI since there is evidence that it crosses the placenta and accumulates in the fetal circulation and in the amniotic fluid.

Antenatal ultrasound imaging remains the major modality for the diagnosis of CAKUT. Melo et al. were able to identify 76% of patients with CAKUT from antenatal ultrasound imaging; however they did not report at what timepoint they were able to establish the diagnosis [41]. Similarly, Policiano et al. were able to establish a prenatal diagnosis of CAKUT in 88.8% of the cases at a median gestational age of 23 weeks (range 11–40 weeks) [42]. While ultrasound remains an excellent method to define the anatomy of CAKUT antenatally, it does not reliably predict postnatal renal function. Fetal renal hyperechogenicity, when the kidneys appear brighter than the liver, can arise from interstitial infiltration, sclerosis, or microcysts in the cortex or in the medulla. In a follow-up of 43 fetuses referred because of bilateral hyperechogenicity, the majority had ARPKD (20 cases), while 8 cases were diagnosed with ADPKD and 7 cases had another CAKUT disorder. The authors concluded that the diagnosis could not be established by prenatal ultrasound in the absence of a family history and that severe oligohydramnios, amniotic pocket less than 2 cm, and large kidney size (>4 standard deviations as seen with ARPKD) were indicative of a poor postnatal renal outcome [43]. Morris et al. performed a systematic review to determine if antenatal ultrasound could predict renal outcome in congenital urinary tract obstruction. Oligohydramnios, renal echogenicity of the parenchyma, or renal cystic change in the cortex were all correlated with poor renal function postnatally, defined as a serum creatinine >106 μmol/L [44]. Many of the studies that were included in the review had limitations as they did not define oligohydramnios.

Oligo/anhydramnios and Consequences

Oligo/anhydramnios from CAKUT is associated with a high incidence of fetal death in utero, severe pulmonary hypoplasia, umbilical cord compression and perinatal asphyxia. The earlier that renal oligohydramnios (ROH) is identified in the pregnancy, the more severe the pulmonary hypoplasia: Klaassen et al. showed that ROH detected before 30 weeks was associated with a higher risk of mortality [45]. Similarly, Mehler et al. showed that non-surviving babies with CAKUT were diagnosed with ROH on average at 25 weeks compared to 31 weeks in survivors [46]. Pulmonary hypoplasia is a disorder of development in which there is a deficiency in the number of lung cells, airways, and alveoli, ultimately leading to a reduced surface area for gas exchange. From post-mortem studies, pulmonary hypoplasia is defined as a decrease in the lung to body weight ratio, <0.12 (67% of mean normal ratio), and a reduction in the radial alveolar counts, <4.1 (75% of mean normal count) [47]. Postnatally, pulmonary hypoplasia can be suspected in cases of ROH when there is respiratory failure with the need for high ventilatory support and the chest x-ray reveals a bell-shaped chest, an elevated diaphragm, and/or pneumomediastinum or pneumothorax. The challenge is how to define pulmonary hypoplasia prenatally to provide accurate information to counsel families on their baby's postnatal renal and respiratory function. While ultrasound and MRI have been used to assess lung volumes antenatally, there is no consensus on whether they are reliable predictors of pulmonary hypoplasia postnatally.

Several fetal biochemical markers have been studied to determine if they predict postnatal renal function. Fetal urine electrolytes including urine sodium, chloride, and urine osmolarity have been assessed, but they lack sensitivity [48]. β2 microglobulin and Cystatin C do not cross the placenta and therefore, fetal blood or urine samples of these biomarkers should correlate with fetal renal function. Spaggiari et al. measured fetal blood β2 microglobulin levels in 34 fetuses and found that a serum level >5 mg/L predicted a poor renal outcome in 87.1% of cases [49]. This is a relatively small sample and in 62% of the cases, the pregnancy was terminated, therefore further studies are needed to validate its use. Most recently, amniotic fluid samples were analyzed from 140 fetuses with CAKUT and this led to the discovery of a 98-peptide signature that correlated with CAKUT [50]. The 98-peptide signature was characterized by the presence of extracellular matrix fragments and the protein known as actin binding protein thymosin-β4. When thymosin-β4 was knocked out in zebrafish, proximal and distal tubular nephron formation was disrupted, suggesting this protein may be a marker of tubule maturation.

Once CAKUT has been identified during the pregnancy, it is important to screen for other organ abnormalities. Melo et al. demonstrated that neonates with CAKUT in addition to chromosomal syndromes, non-chromosomal syndromes, VATER, or any organ defect had a much higher risk of neonatal mortality (Odds ratio 20.7, 95% confidence interval) [41]. The same group also found that fetuses diagnosed with CAKUT and multiple malformations and chromosomal defects had a neonatal mortality rate of 76% [51]. Indeed if other organ morbidities are identified or there is oligohydramnios, then it is imperative that there is shared decision making between the parents and health care team regarding the implications of these findings to establish a plan of action for either termination of pregnancy, or palliation at the time of birth, or treatment [52].

When isolated CAKUT or CAKUT with other organ defects is suspected on the antenatal ultrasound, genetic counseling and referral to a multidisciplinary team should be offered. As outlined by Talati et al., an individualized approach that is in accordance with the parental wishes for more information is advised [53]. A screen for gross chromosomal abnormalities using a chromosomal microarray can be performed from the amniotic fluid (if less than 25 weeks) or from fetal blood (>than 25 weeks or severe oligohydramnios). If the chromosomal microarray is normal, then a targeted gene panel or whole exome sequencing (WES) on both parents and the fetus could be considered. Large cohort studies of affected fetuses are needed to clarify the benefit of WES or targeted gene panels for prenatal diagnosis and treatment.

In the setting of severe CAKUT with and without other organ defects, termination of the pregnancy must be considered and discussed with the parents. In most cases, the findings from ultrasound will provide sufficient information to guide decision-making. Legal limits for when termination of pregnancy can occur vary between countries, and sometimes even within countries, there are regional differences for when this can occur during gestation. These are difficult discussions and when available, a palliative care team can be beneficial to provide support to the family and the treating team. If there is a neonatal demise or a termination of pregnancy, it can be informative to perform an autopsy and obtain DNA for genetic testing to inform the parents about their risk for future pregnancies.

Treatment of Congenital Anomalies of the Kidney with Oligohydramnios

CAKUT with renal oligohydramnios due to lower urinary tract obstruction or renal agenesis has a poor outcome with high mortality. Vesicoamniotic shunting has been used as a treatment for CAKUT with lower urinary tract obstruction and may have benefit. Clearly, this type of intervention is of no benefit if oligo/anhydramnios is secondary to bilateral renal agenesis or severe renal hypodysplasia with decreased fetal urine production. Serial amnioinfusions have been used to treat fetuses with no or low fetal urine production to palliate pulmonary hypoplasia and reduce the risk of newborn mortality. A recent review of serial amnioinfusions identified a total of 22 fetuses that have been treated: 7 with lower urinary tract obstruction, 8 with congenital renal anomalies, and 7 with lower urinary tract obstruction and congenital renal anomalies [54]. The median number of amnioinfusions was 7 and the median time for the first infusion was at 23 weeks gestation. Impressively, the median age of newborns at delivery was 32 weeks gestation. Of the 22 neonates, 11 survived for at least 1 month, 16 received peritoneal dialysis and 9 went on to receive a renal transplant. Importantly 8 did not require intubation and survived at least until 1 month of age. At present, there is a need for further research to demonstrate efficacy and beneficence.

Genetics of Congenital Kidney Anomalies

Complex Phenotypes from Variable Genetic Backgrounds

From a clinical perspective, determining the etiology of developmental defects can result in more precise diagnostic classification and in the future, this may drive therapeutic strategies to change the clinical outcome of these disorders [55]. The advent of next generation sequencing techniques has redefined the molecular landscape of congenital kidney defects over the last two decades. Next generation sequencing (NGS) approaches are based on automated massive parallel sequencing using clonally amplified DNA templates, allowing quick assessment of an entire human genome. Before the introduction of next generation sequencing, Sanger sequencing (i.e., individual sequencing reactions) of the locus of interest was primarily used for gene discovery in human diseases, but the short reads of <1 kb per sequence and the low throughput of this technique limited testing of multiple variants at once. Nevertheless, most genetic mutations that cause congenital kidney anomalies have been discovered using Sanger sequencing.

Genetic studies of congenital kidney malformations have resulted in the identification of more than 50 genes that have been implicated in syndromic (complex) or isolated forms of congenital kidney defects (Table 3) [55]. While this number continues to grow, a recent report has indicated that there is an extremely high rate of pathogenic variants in individuals self-reported to be healthy and without kidney disease [56]. This suggests that there is a need for more stringent curation of variants and long-term follow-up of individuals with these variants to avoid genetic misdiagnosis.

Many disease-causing genes in congenital kidney anomalies encode transcription factors or signaling molecules that are required for the interaction between the ureteric bud and metanephric mesenchyme during kidney development (see also ▶ Chap. 1, "Pre-natal Development of the Kidneys and Urinary Tract"). There is tremendous heterogeneity in the etiology of congenital kidney anomalies. In addition, incomplete or non-penetrance of gene mutations is commonly observed in affected families. Identical pathogenic mutations can result in completely different CAKUT phenotypes, even between individuals within the same family. Taken together, there is variable expressivity of gene mutations implicated in CAKUT. As noted previously, co-occurrence of urinary tract defects including obstructive uropathy, VUR, or lower urinary tract obstruction is frequently seen with congenital kidney defects. For example, 20–30% of individuals with unilateral renal agenesis or MCDK have a urinary tract malformation [8, 13]. It is not clear what causes the genetic and phenotypic variation of CAKUT, but one can hypothesize that other genetic factors like oligo- or polygenic inheritance, epigenetic changes and/or environmental factors contribute to the variation [57].

Table 3 Glossary of genes associated with congenital kidney defects

OMIM	Gene symbol	Gene product	Gene/Protein family and function	Extrarenal findings/syndrome diagnosis
#106180	*ACE*	Angiotensinogen	Renin substrate	Renal tubular dysgenesis
#106150	*AGT*	Angiotensin-converting enzyme	Zinc metalloproteinase enzyme	Renal tubular dysgenesis
#106165	*AGTR1*	Angiotensin II receptor type 1	GPCR	Renal tubular dysgenesis-
#617298	*AIFM3*	Apoptosis-inducing factor 3	Oxidoreductase	DiGeorge syndrome
#112262	*BMP4*	Bone morphogenetic protein 4	TGF-beta	Cleft lip, microphthalmia
#608892	*CHD7*	Chromodomain helicase DNA binding protein 7	SNF2/RAD54 helicase	CHARGE
#613039	*CHD1L*	Chromodomain helicase DNA binding protein 1 like	SNF2/RAD54 helicase	Cryptorchidism
#600856	*CDKN1C*	Cyclin dependent kinase inhibitor 1C	CDI family	Beckwith-Wiedemann
#118494	*CHRM3*	Cholinergic receptor muscarinic 3	Muscarinic acetylcholine receptor	Prune-Belly-like phenotype
#600140	*CREBBP*	CREB binding protein	Histone acetyltransferase	Rubinstein-Taybi
#602007	*CRKL*	CRK-like protein	Protein kinase	DiGeorge syndrome
#602858	*DHCR7*	7-Dehydrocholesterol reductase	ERG4/ERG24 family, cholesterol biosynthesis	Smith-Lemli-Opitz
#612666	*DSTYK*	Dual serine/threonine and tyrosine protein kinase	Protein kinase superfamily	Seizures (?)
#600855	*DYRK1A*	Dual specificity tyrosine phosphorylation regulated kinase 1A	Protein kinase superfamily	Intellectual disability
#601653	*EYA1*	Eyes absent homolog 1	EYA1 proteins	Branchio-oto-renal
#605558	*FGF20*	Fibroblast growth factor 20	Heparin-binding growth factors family	–
#136350	*FGFR1*	Fibroblast growth factor receptor 1	Protein kinase superfamily	Hypogonadotropic hypogonadism with or without anosmia
#601090	*FOXC1*	Forkhead box C1	Forkhead family of transcription factors	Axenfeld-Rieger syndrome,
#605515	*FOXP1*	Forkhead box P1	Forkhead family of transcription factors	Structural brain abnormalities, seizures, skeletal abnormalities
#607830	*FRAS1*	Fraser syndrome 1	Extracellular matrix protein	Fraser
#608944	*FREM1*	Fraser syndrome 2	Extracellular matrix protein	Fraser
#608945	*FREM2*	FRAS1-related extracellular matrix 2	Extracellular matrix protein	Fraser
#131320	*GATA3*	GATA3	Transcription factor with GATA zinc-fingers	Hypothyroidism, sensorial deafness

(continued)

Table 3 (continued)

OMIM	Gene symbol	Gene product	Gene/Protein family and function	Extrarenal findings/syndrome diagnosis
#146255	*GLI3*	GLI family zinc finger 3	Transcription factor	Pallister-Hall
#617782	*GREB1L*	Greb1-like retinoic acid receptor coactivator	GREB1 family	Mayer-Rokitansky-Kuster-Hauser (presumed)
#300037	*GPC3*	Glypican 3	Cell surface heparin sulfate proteoglycan	Simpson-Golabi-Behmel
#605310	*7-HCR*	Alpha-helix coiled-coil	Role in DNA repair and	–
#189907	*HNF1β*	Hepatocyte nuclear factor 1-beta	Homeobox transcription factor	Renal cysts and diabetes (RCAD)
#611177	*IFT80*	Intraflagellar transport 80	Sensory cilia motility factor	Jeune syndrome
#601920	*JAG1*	JAGGED1	Notch receptor ligand	Alagille
#300836	*KAL1 or ANOS1*	Anosmin-1	Regulates migration of nerve cell precursors	Hypogonadotropic hypogonadism with or without anosmia
#602113	*KMT2D*	Lysine methyltransferase 2D	Histone-lysine methyltransferase family	Kabuki
#300128	*KDM6A*	Lysine demethylase 6A	Lysine-specific demethylase	Kabuki
#151443	*LIFR*	LIF receptor subunit alpha	type I cytokine receptor family	Stuve-Wiedemann syndrome/ Schwartz-Jampel type 2 syndrome
#142994	*MNX1*	Motor neuron and pancreas homeobox 1	Homeobox domain transcription factor	VACTERL/ Caudal regression / Currarino
#6002756	*NOTCH2*	Notch receptor 2	Notch family of transmembrane proteins	Alagille
#608667	*NIPBL*	NIPBL cohesin loading factor	Cohesin loading onto chromatids	Cornelia de Lange
#602490	*NRIP1*	Nuclear receptor interacting protein 1	Modulates transcriptional activity of the estrogen receptor	
#600856	*P57KIP2*	Cyclin-dependent kinase	Cell cycle inhibitor	Beckwith-Wiedemann
#167409	*PAX2*	Paired box 2	Transcription factor	Papillorenal syndrome
#176310	*PBX1*	Pre-B cell leukemia transcription factor	Transcription factor	CAKUT, hearing loss, abnormal ears, or developmental delay
#214100	*PEX1*	Peroxin 1	Peroxisomal ATP-ases	Zellweger
#179820	*REN*	Renin	Enzyme that cleaves AGT to produce Ang I	Renal tubular dysgenesis
#602218	*SALL1*	Sal-like 1	Zinc finger transcription	Townes-Brocks
#607343	*SALL4*	Sal-like 4	Zinc finger transcription	Duane-radial ray
#603961	*SEMA3A*	Semaphorin 3A	Semaphorin family	Hypogonadotropic hypogonadism with or without anosmia
#601205	*SIX1*	Sine oculis homeobox 1	Homeobox transcription factors	Branchio-oto-renal

(continued)

Table 3 (continued)

OMIM	Gene symbol	Gene product	Gene/Protein family and function	Extrarenal findings/syndrome diagnosis
#604994	*SIX2*	Sine oculis homeobox 2	Homeobox transcription factors	–
#600963	*SIX5*	Sine oculis homeobox 1	Homeobox transcription factors	Branchio-oto-renal
#604202	*SNAP29*	Synaptosome associated protein 29	Membrane trafficking	Cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma syndrome / DiGeorge
#608160	*SOX9*	Sex determining region Y-box 9	HMG-box class of DNA binding proteins	Campomelic dysplasia
#600898	*SOX11*	Sex determining region Y-box 9	HMG-box class of DNA binding proteins	Coffin-Siris
#606523	*SRGAP1*	SLIT-ROBO Rho GTPase activating protein 1	GTPase activator	Cleft palate, developmental delay
#609850	*TBC1D1*	TBC1 domain family member 1	GTPase-activating protein	Insulin resistance
#601621	*TBX3*	T-box 3	T-box transcription factor	Ulnar-Mammary
#602427	*TBX6*	T-box 6	T-box transcription factor	Spondylocostal dysostosis
#606219	*TRAP1*	TNF receptor associated protein 1	Heat shock protein 90	VACTERL
#611559	*UPK3A*	Uroplakin 3A	Component of the asymmetric unit membrane	–
#610132	*VANGL1*	VANGL planar cell polarity protein like 1	Vang family	VACTERL / Caudal regression
#603490	*WNT4*	Wnt family member 4	Wnt family of signaling proteins	Mullerian aplasia and hyperandrogenism
#602221	*ZMYM2*	Zinc finger MYM-type containing 2	Zinc finger transcription factor	Skeletal abnormalities, cardiac defects, intellectual disability, dysmorphic facial features

OMIM Online Mendelian Inheritance in Man, *GPCR* G protein-coupled receptor, *CREB* cAMP response element-binding, *HMG* High-Mobility Group, *Ang* Angiotensin

Genetic Perspective on Congenital Kidney Anomalies

Our knowledge of the molecular etiology of congenital kidney anomalies is based on their familial occurrence and the fact that patients with some genetic mutations have a CAKUT phenotype that correlates with a genetically defined animal model. However, the heritability of congenital kidney defects (i.e., the proportion of affected individuals in which genetic factors are causal) remains unknown and systematic studies to estimate heritability have not been performed. Currently, we can establish a molecular diagnosis in 5–30% of affected individuals, but this is highly dependent on the characteristics of the study cohort. The genetics of congenital kidney anomalies can be summarized by three distinct molecular origins:

1. *Rare single nucleotide variants* (SNVs) with large effects cause monogenic disease (Mendelian disorders), i.e., one coding base pair change in a particular gene is deleterious and causes the phenotype. Pathogenic SNVs in CAKUT predominantly follow an autosomal dominant mode of inheritance, however, autosomal recessive and X-linked modes of inheritance have been identified.

2. *Large rare structural variants* (copy number variants or CNVs) have been implicated in 5–10% of isolated and syndromic forms of congenital kidney anomalies. Pathogenic deletions or duplications of multiple base pairs may involve entire chromosomes, multiple genes, or parts of genes that disrupt normal kidney development.
3. *Common variants* (generally caused by non-coding single nucleotide polymorphisms [SNPs]) usually have small effects and have not yet been elucidated in congenital kidney anomalies due to the absence of large cohorts of patients. The oligo/polygenic landscape of congenital kidney defects assumes that multiple genetic defects together give rise to the phenotype. This molecular mechanism is largely unexplored because it requires the integration of genome-wide sequencing data from 10,000 s of patients. However, for other common diseases such as diabetes and inflammatory bowel disease, this approach has been successfully utilized.

Monogenic Disorders

The monogenic basis of congenital kidney anomalies is founded on several observations: (1) Congenital kidney anomalies occur within families, frequently following Mendelian autosomal dominant inheritance patterns. The study of these families has contributed to our current understanding of the genetic basis of congenital anomalies of the kidney and urinary tract. (2) Sporadic kidney malformations often occur as part of a specific genetic syndrome in which multiple developmental defects occur simultaneously in an affected individual (see below). These syndromes are predominantly explained by de novo heterozygous variants with large effects on amino acid translation or heterozygous deletions that disrupt multiple genes. These cases arise sporadically or de novo because the affected individuals often have reduced reproductive fitness, which demonstrates that these variants are under strong selective pressure. The analysis of syndromic disorders characterized by extrarenal manifestations including ocular coloboma, preauricular tags or fistulas, and anorectal malformations in addition to CAKUT, led to the discovery of *HNF1β* (renal cyst and diabetes, OMIM, #137920)*,* *PAX2* (papillorenal syndrome, #120330)*,* *EYA1* (branchio-oto-renal syndrome, #113650), *SALL1* (Townes-Brocks syndrome, #107480), *WT1* (Denys-Drash syndrome, #194080), and others as “syndromic genes.” Follow-up studies have subsequently identified pathogenic variants in the same genes in isolated (non-syndromic) forms of congenital kidney malformations. Homozygous recessive mutations occur predominantly in consanguineous families or from a historical founder effect in isolated populations. Compound heterozygous mutations and X-linked inheritance have also been identified in congenital kidney malformations. Finally, The fact that single-gene knockouts in model organisms (i.e., *Drosophila*, zebrafish and mice) recapitulate the human CAKUT phenotype is further evidence of the causal role of monogenic disease in human kidney defects [57].

The evidence that congenital kidney anomalies are a monogenic disorder was systematically demonstrated in the ESCAPE cohort study in 2006 [58]. Using Sanger sequencing of five candidate genes (i.e., *HNF1β (synonym TCF2)*, *PAX2*, *EYA1*, *SIX1*, and *SALL1*), Weber et al. identified 17 (17%) pathogenic mutations in 100 unrelated families. Of these, 15 were found in *HNF1β* or *PAX2*. Currently, mutations in these genes are still the most common genetic cause of congenital kidney anomalies. Another important finding from the Weber study was that after the genetic diagnosis was established, clinical re-evaluation and family analysis revealed extrarenal syndromic features in 9 of 17 patients. This highlights the additional clinical value of genetic testing.

Mutations of *HNF1β* were initially identified in adults with a genetic form of diabetes (maturity onset of diabetes in the young (MODY) type 5) who were subsequently found to have cystic kidney disease [59]. Individuals with so-called renal cyst and diabetes syndrome (RCAD) typically harbor a pathogenic mutation in *HNF1β* or they carry a microdeletion of chromosome 17q12, which encompasses *HNF1β* (see below). Since its genetic discovery in 1997, the phenotypic spectrum of *HNF1β* mutations has expanded enormously. It includes the entire spectrum of

CAKUT, disorders of pancreatic development including endocrine and exocrine dysfunction, maldevelopment of the female and male genital tracts, neonatal cholestasis and elevated liver enzymes, primary hyperparathyroidism, hyperuricemia, gout, renal magnesium wasting, and sensorineural deafness (see also ▶ Chap. 53, "Autosomal Dominant Tubulointerstitial Kidney Disease"). Approximately 2–30% of affected individuals with congenital kidney anomalies carry pathogenic mutations in *HNF1β* or the microdeletion of chromosome 17q12. In patients with renal cysts and MODY, the prevalence of deleterious mutations in *HNF1β* is even higher at 40% [60]. *HNF1β* has also been widely recognized as the most common monogenic cause of congenital kidney anomalies, particularly in MCDK. Because of the highly variable phenotypes and incomplete penetrance observed in individuals with mutations in *HNF1β*, an *HNF1β* score has been developed based on clinical findings, imaging and biological variables to identify who should undergo genetic testing. A score greater than 8/48 is strongly associated with the identification of a mutation in *HNF1β* [61].

The transcription factor PAX2 is relevant during kidney and eye development. Along with PAX6, PAX2 coordinates the development of the optic vesicle. Sanyanusin et al. were the first to discover a pathogenic mutation in *PAX2* in a family with optic nerve colobomas, kidney hypoplasia, proteinuria, and vesicoureteral reflux [62]. Mutations in *PAX2* lead to congenital kidney defects with or without ophthalmologic manifestations such as retinal coloboma and microphthalmia. Since its initial discovery, the phenotypic spectrum of mutations in *PAX2* has expanded to include sensorineural deafness, skeletal deformities, ovarian teratoma, renal carcinoma, gout, and ventricular septal defects. Vivante et al. reported a heterozygous mutation in *PAX2* in children with steroid-resistant nephrotic syndrome and focal segmental glomerulosclerosis (FSGS) [63], which may be consistent with another group that identified heterozygous mutations in *PAX2* in patients with adult-onset FSGS. Although the potential pathogenic mechanism is unknown, it is plausible that mutations in *PAX2* result in insufficient nephron progenitor cell differentiation and a deficit in nephron formation. This may, in turn, result in hyperfiltration injury with overt proteinuria and FSGS [63]. It is compelling to consider that low nephron number at birth due to a mutation in a gene that is required for kidney development may be at the root of many cases of adult-onset FSGS, hypertension and/or renal failure.

More recently, whole exome sequencing has identified truncating dominant mutations in *GREB1L* (encoding *growth regulation by estrogen in breast cancer 1-like*) as a novel monogenic cause of severe congenital kidney malformations, including bilateral kidney agenesis. When *Grebl1* was knocked out in zebrafish or in the mouse, the kidneys failed to form [64, 65]. In a subset of females with pathogenic mutations in *GREB1L*, developmental defects of the uterus (i.e., agenesis of the uterus, unicornuate uterus) consistent with Mayer-Rokitansky-Kuster-Hauser syndrome have been identified, whereas others only exhibited isolated congenital kidney malformations [65, 66]. *GREB1L,* therefore, has a role during Mullerian organogenesis. The exact function of GREB1L is unknown, but it may function as a coactivator of retinoic acid receptors.

Autosomal recessive inheritance of congenital kidney anomalies is seen with the genes that are implicated in Fraser syndrome. *FRAS1, FREM1, FREM2,* and *GRIP1* all encode extracellular matrix proteins which play a role in cell-cell and cell-matrix interactions between the metanephric mesenchyme and the ureteric bud epithelium. Homozygous truncating mutations in the Fraser complex genes result in Fraser syndrome which encompasses congenital kidney malformations, eye defects, and additional extrarenal features. Interestingly, biallelic missense mutations in these genes have been identified in isolated kidney defects. This is a good example of the impact of specific gene mutations on protein synthesis. Missense mutations result in a full-length protein in which a specific amino acid has been modified (presumably leading to decreased but not absent function) and this results in an isolated kidney defect. In contrast, truncating mutations result in no protein synthesis and this leads to a more extensive phenotype that includes both the renal and the extrarenal features of Fraser syndrome.

Groopman et al. have demonstrated the diagnostic value of next generation sequencing in monogenic kidney disease in 3,037 adults with chronic kidney disease of various etiologies [67]. The authors found the highest diagnostic yield in patients with congenital or cystic kidney disease (23.9%; OR 24.4, 95% confidence interval 10.6–56.4 compared to a reference group of diabetic nephropathy). In a smaller cohort of adults with chronic kidney disease, monogenic causes of CAKUT were identified in 14 out of 114 (12.2%) families containing 138 affected individuals [68]. The variability in diagnostic yield of targeted exome-sequencing studies in specific populations with congenital kidney anomalies is strongly associated with the characteristics of the screening population. For example, while deleterious variants were identified in 20–30% of fetuses with prenatally diagnosed kidney anomalies [69], Nicolaou et al. identified a much smaller contribution of monogenic disorders in a more heterogeneous group of children with CAKUT than expected (6 causal mutations in 453 screened patients, 1.3%) [70]. The yield of genetic screening in children with congenital kidney anomalies will therefore vary based on the phenotype of the cohort, the control population that is used to assess sequence variants and the filtering pipeline that is used to prioritize variants. Targeted gene panels are an important diagnostic tool for cost-effective analysis of the molecular etiology of genetic kidney diseases, however, they have limitations. They do not take into account the possibility of phenocopies (i.e., the phenotype is caused by environmental conditions rather than genetic factors), phenotypic expansion of disease (i.e., the phenotype has not been previously attributed to the disease gene, and hence, is not included in the gene panel) and/or the chance of coincidental diagnosis, meaning the discovery of a gene mutation that is unrelated to the phenotype. For these reasons, it may be more prudent to use whole exome or genome sequencing to evaluate all genes collectively [71]. Of benefit, these more comprehensive genetic tests can be re-analyzed with updated knowledge of pathogenic mechanisms at any given time-point.

Copy-Number Variation

Besides monogenic disorders, congenital kidney anomalies can be attributed to copy-number variants (CNVs), which are defined as any gain or loss of germline DNA ranging from 1,000 basepairs to entire chromosomes (i.e., megabases [Mb]). CNVs influence gene expression, phenotypic variation within the general population, and result in imbalances in gene dosage and/or disruption of genes. CNVs below 1 to 2 Mb are usually not detectable by conventional cytogenetic techniques, but they are readily detected by chromosomal microarrays (array comparative genomic hybridization or SNP arrays), which are the preferred technique for detection of genomic imbalances. CNVs which occur more often in people with the disease of interest are defined as recurrent CNVs. Recurrent CNVs are driven by highly repetitive elements in the human genome and result in recurrent breakpoints in affected individuals. Non-recurrent CNVs have variable breakpoints and are driven by inadequacies in DNA repair mechanisms. There are over 200 recurrent CNVs that underlie human congenital malformations such as neurocognitive disorders, cardiac anomalies, craniofacial malformations, neuropsychiatric disorders and CAKUT. Golzio and Katsanis have proposed a simplex model in which a single gene imbalance within the CNVs is responsible for the genomic disorder (i.e., the chromosome 17q12 microdeletion containing *HNF1β*), and a more complex model in which multiple modifier genes within and/or outside the CNVs determine the constellation of developmental phenotypes [72]. Like syndromic CAKUT caused by single gene defects, pathogenic CNVs display incomplete penetrance and variable expressivity of disease.

Genomic disorders are diseases that are characterized by loss or gain of CNVs. Sanna-Cherchi and Gharavi analyzed a cohort of 522 individuals with kidney hypodysplasia using SNP-arrays and discovered that cases had a much higher burden of large and rare exonic CNVs than controls [73]. In addition, they identified known genomic disorders in 10.1% of patients and potentially pathogenic CNVs in 6.1% of patients. Not surprisingly, the most prevalent genomic disorders were the chromosome 17q12 microdeletion (RCAD locus) and the chromosome 22q11.2 microdeletion (DiGeorge syndrome locus). However, a substantial fraction of known genomic disorders occurred in cases without extrarenal features, supporting the fact that CNVs also exhibit variable expressivity [73].

In a smaller cohort of 80 children with congenital kidney malformations, this higher burden of large and rare CNVs was replicated in cases compared to population controls [74]. Under the assumption of a simplex model in which one gene causes the CNV phenotype, a systematic in silico approach identified *DLG1* and *KIF12* as potential genetic drivers of congenital kidney malformations.

The same team of investigators expanded CNV analyses to the entire spectrum of CAKUT phenotypes ($n = 2{,}824$) of which congenital kidney malformations (kidney hypodysplasia) were the largest group ($n = 1{,}088$) [75]. The authors found that large and rare CNVs were most prevalent in isolated and syndromic forms of congenital kidney malformations when compared to over 21,000 healthy controls (CNVs ≥500 kb OR 2.20, 95% confidence interval 1.79–2.58; *P*-value 5.67×10^{-15}). Interestingly, the enrichment of genomic imbalances was much less pronounced in children with duplex kidneys and even absent in the case of ectopic kidney tissue, suggesting a different molecular basis for these anomalies. Overall, a genomic disorder was diagnosed in 7.3% of individuals with congenital kidney malformations (OR 12.65, 95% confidence interval 9.40–16.94; *P*-value: 8.53×10^{-50} vs. population-based controls). Although unique CNVs occurred, most of the genomic disorders were identified at the following chromosomal locations: 17q12 locus (RCAD), 22q11.2 (DiGeorge), 4p1 (Wolf-Hirschhorn), 16p11.2 and 1q21.1 [75]. In contrast to other CAKUT phenotypes, congenital kidney anomalies were predominantly caused by deletions at the abovementioned loci and not by duplications. This suggests that congenital kidney defects predominantly originate from haploinsufficiency, i.e., a single copy of the wild-type allele is insufficient for formation of normal kidney tissue, or a dominant negative effect in which disruption of a gene adversely affects the normal, wild-type gene product. Approaching the common genomic disorders in congenital kidney anomalies from a one driver gene model has resulted in increasing insight into the molecular etiology of disease. For the chromosome 17q12 locus *HNF1β* is the major driver. For the chromosome 22q11.2 locus, *CRKL* appears to be the major genetic driver for congenital kidney malformations, however the adjacent genes, *AIFM3* and *SNAP3,* may also be implicated [76]. For the chromosome 16p11.2 locus, which is the most prevalent cause of autism spectrum disorder, *KCTD13* has been implicated as the driver of the neurocognitive phenotype [77]. In contrast, the transcription factor *TBX6* has been proposed as the candidate gene driver for the congenital kidney malformations associated with the 16p11.2 microdeletion [75]. This was based on the finding that individuals with mutations in *TBX6* have kidney hypodysplasia. Similar findings were recapitulated in mice with a null mutation in *Tbx6 [75]*. Another group proposed that the transcription factor *MAZ* that regulates urogenital development is the main genetic driver for the kidney phenotype in the 16p11.2 microdeletion [78]. It is possible that a more complex genetic etiology underlies the kidney malformations associated with this genomic disorder.

From a clinical perspective, CNVs are relevant for antenatal diagnostic purposes for two reasons: (1) as highlighted earlier in this chapter, the majority of affected individuals with congenital kidney malformations are now identified in utero, and (2) the a priori chance of finding a genetic etiology for a developmental kidney defects is higher when extrarenal malformations are present. Recent studies in children with a fetal diagnosis of multicystic dysplastic kidney and renal hypodysplasia reported a diagnostic yield of pathogenic CNVs of approximately ~12.5%. Furthermore, Sanna-Cherchi et al. noted substantial overlap with known CNVs causing neurodevelopmental defects and psychiatric disorders, such as autism and schizophrenia [55]. This suggests that children with congenital kidney defects may be at increased risk of intellectual disability as well as other neurocognitive defects. Indeed, subsequent analysis of the Chronic

Kidney Disease in Children (CKiD) study cohort demonstrated that children with pathogenic CNVs had significantly lower neurocognitive scores for domains such as IQ, depression/anxiety, and executive functioning [79]. In summary, chromosomal microarrays should be considered a first line diagnostic test for children with congenital kidney anomalies [80].

Common Variants

Most congenital kidney malformations arise from rare monogenic disorders caused by non-synonymous coding variants with large effects. Common variants that generally consist of non-coding or synonymous coding variants, typically have small effect sizes and therefore much larger cohorts of patients are required to infer causality. Common variants are usually identified by genome-wide association studies (GWAS) using SNP arrays. Since their introduction in the early 2000s, chromosomal microarrays have identified many loci in which the DNA sequence of individuals with kidney disease differs from population-based controls. However, only a handful of these loci have been directly linked to disease causality. This is because often, their biological function is poorly understood, functional validation studies are absent, and/or large differences in allele frequencies exist between populations. These substantial hurdles have limited the use of GWAS to understand congenital kidney malformations. Duplex kidneys and ectopic kidneys may have a genetic background based on common variants given their relatively high prevalence in the population. Knowledge of common variants is an important missing piece in the etiology of congenital kidney malformations. It is conceivable that CAKUT could arise from the combined effects of thousands of common variants at once. By integrating genetic methods, functional modeling of disease, bioinformatic tools and statistical genetic analyses, common variants identified from GWAS can drive the generation of oligo- or polygenic risk scores (PRS). PRS combine the sum of all known variants to calculate an overall risk of developing a particular disease. This approach has been successfully used to understand diseases such as coronary artery disease, inflammatory bowel disease, and diabetes mellitus, and could be applied to better understand CAKUT [81].

Syndromes Associated with CAKUT

Syndromes are characterized by a set of associated signs and symptoms where a genetic cause is known. Human CAKUT is frequently observed as part of a genetic syndrome (Table 4). Importantly, syndromes that are due to ciliopathies like nephronophthisis can phenocopy CAKUT and with the exception of Jeune syndrome are not included in this list (see ▶ Chap. 52, "Nephronophthisis and Related Ciliopathies").

Alagille syndrome (AS; OMIM #118450) is an autosomal dominant disorder characterized by a paucity of interlobular bile ducts, chronic cholestasis, cardiac anomalies, butterfly vertebrae, corneal abnormalities, dysmorphic facies, and cystic renal dysplasia [82]. AS is due to mutations in Jagged-1 *(JAG1)*, which encodes a ligand for the Notch receptor. Jagged ligand/Notch receptor interactions between cells are critical for determination of cell fates during development. Early in kidney development, Notch signaling regulates nephron and collecting duct cell differentiation. Notch signaling represses expression of SIX2 in nephron progenitor cells (NPCs), which causes premature differentiation of NPCs and results in less nephron formation [108]. In collecting duct cells, activated Notch signaling promotes principal cell differentiation at the expense of intercalated cell fates [109]. During the later stages of nephron morphogenesis, loss of Notch signaling promotes the formation of proximal tubular cysts, an increase in epithelial cell proliferation, and renal papillary microadenomas.

Apert syndrome (acrocephalosyndactyly type I, OMIM #101200) is an autosomal dominant disorder characterized by craniofacial defects, maxillary hypoplasia, protruding eyes, hypertelorism, and hydronephrosis [83]. It occurs in 6 to 15.5 out of one million livebirths. Most cases result from activating mutations in the gene encoding *fibroblast growth factor* (*FGF*) *receptor 2* (*FGFR2*), located on chromosome 10 [83]. The FGF family consists of 22 structurally related proteins and plays a central

Table 4 Multiorgan syndromes associated with CAKUT in humans

Syndrome	OMIM	MOI	Gene	Extrarenal phenotype	Renal phenotype	References
Alagille	118450	AD	*JAGGED1*	Intrahepatic cholestasis abnormalities of heart, eyes, and vertebrae	Cystic dysplasia	[82]
Apert	101200	AD	*FGFR2*	Craniosynostosis, syndactyly	Hydronephrosis	[83]
Beckwith-Wiedemann	130650	AD	*p57KIP2,* *IGF2,* *KCNQ1,* *KCNQ1OT1*	Macroglossia, gigantism, visceromegaly	Medullary dysplasia Wilms' tumor	[84]
Branchio-Oto-Renal	113650	AD	*EYA1* *SIX1* *SIX5*	Deafness, ear malformations, branchial cysts	Unilateral or bilateral renal agenesis, VUR renal hypodysplasia	[85, 86]
CAKUTHED	617641	AD	*PBX1*	Deafness, ear malformations developmental delay	Renal hypodysplasia, Horseshoe kidney, cystic dysplasia, VUR	[87]
Campomelic dysplasia DiGeorge	114290 188400	AD	*SOX9* *TBX1* *CRKL* *SNAP29* *AIFM3*	Short and bowed femora and tibiae, hypoplastic vertebrae cranial anomalies, heart defects Cardiac anomalies, thymic hypoplasia, hypocalcemia, hypoparathyroidism	Hydroureter, renal hypodysplasia hydronephrosis Unilateral or bilateral renal agenesis, renal dysplasia, hydronephrosis	[88, 89] [76, 90]
Fraser	219000	AR	*FRAS1,* *FREM1* *FREM2,* *GRIP1*	Cryptophthalmos, syndactyly, ear, and heart defects	Renal agenesis, dysplasia	[91]
HDR	146255	AD	*GATA3*	Hypoparathyroidism sensorineural deafness	Renal dysplasia	[92, 93]
Jeune	611177	AR	*IFT80*	Skeletal dysplasia with small thorax	Cystic kidney disease	[94]
Kallman	300338	XL	*KAL1 or ANOS1*	Anosmia, hypogonadotrophic hypogonadism	Renal agenesis	[95, 96]
Mammary-ulnar	181450	AD	*TBX3*	Posterior limb deficiencies hypoplastic teeth and breasts delayed pubertal development	Renal dysplasia	[97]
Mayer-Rokitansky-Kuster-Hauser	277000	AD	*GREB1L*	Uterovaginal aplasia, primary amenorrhea, short neck, Klippel-Feil deformity of cervical spine	Unilateral or bilateral renal agenesis	[66, 98]
Okihiro	607323	AD	*SALL4*	Limb, anorectal, ear, heart, eye movement abnormalities	Renal agenesis, VUR cross-fused ectopia	[99]
Pallister-Hall	146510	AD	*GLI3*	Polysyndactyly, hypothalamic hamartoma, tracheal stenosis imperforate anus	Renal dysplasia	[100]

(continued)

Table 4 (continued)

Syndrome	OMIM	MOI	Gene	Extrarenal phenotype	Renal phenotype	References
Renal-coloboma	120330	AD	*PAX2*	Optic nerve coloboma hearing loss	Renal hypoplasia, VUR	[62]
Renal cysts and diabetes	137920	AD	*HNF1β*	MODY, genital anomalies	Glomerulocystic kidney disease, MCDK, uni- or bilateral cystic kidneys renal hypodysplasia horseshoe kidney oligomeganephronia	[101]
Renal tubular dysgenesis	267430	AR	*AGT* *REN* *ACE* *AT1R*	Oligohydramnios, facial dysmorphia, arthrogryposis lung hypoplasia, skull ossification defect	Tubular dysplasia	[102]
Rubenstein-Taybi	180849	AD	*CREBBP,* *EP300*	Mental retardation, heart defects broad thumbs and toes	Renal hypolasia	[103]
Simpson-Golabi-Behmel	312870	XL	*GPC3*	Somatic overgrowth, tumors	Medullary dysplasia	[104]
Smith-Lemli-Opitz	270400	AR	*DHCR7*	Microcephaly, mental retardation micrognathia, ptosis of eyelids poly/syndactyly, cataracts heart defects	Renal agenesis, dysplasia	[105]
Townes-Brocks	107480	AD	*SALL1*	Anal atresia, sensorineural hearing loss, triphalangeal thumbs	Renal hypodysplasia, VUR	[106]
Zellweger	214100	AR	*PEX1*	Dysmorphic facial features neurologic dysfunction	Cystic dysplasia, VUR	[107]

Please see Table 1 for glossary of genes
AD Autosomal dominant, *AR* Autosomal recessive, *MOI* Mode of Inheritance, *XL* X-linked, *MODY* Maturity-onset diabetes of the young, *MCDK* Multicystic dysplastic kidney, *VUR* Vesico-ureteral reflux

role in the growth and differentiation of mesenchymal and neuroectodermal cells. Studies in mice demonstrate an important role for FGFR2 in maintaining NPCs, ureteric bud (UB) branching, and ureter and bladder mesenchymal development [110].

Beckwith-Wiedemann syndrome (BWS; OMIM #130650) is a pediatric overgrowth disorder characterized by a predisposition to tumor development. BWS results from dysregulation of imprinted gene expression in the chromosomal region 11p15.5 and in genomic loci outside of this region [84]. The critical BWS genes in the 11p15.5 region include *cyclin-dependent kinase inhibitor 1C* (*CDKN1C/p57KIP2*) that regulates cell cycle progression, *insulin-like growth factor 2* (*IGF2*), *potassium channel voltage-gated KQT-like subfamily member 1* (*KCNQ1*), and *KCNQ1-overlapping transcript 1* (*KCNQ1OT1*, or *long QT intronic transcript 1*). Clinical manifestations of BWS include macrosomia, visceromegaly, macroglossia, hemihyperplasia, omphalocele, ear lobe creases, posterior helical ear pits, and embryonal tumors including Wilms' tumors, hepatoblastomas, and neuroblastomas. Renal abnormalities include congenital anomalies of the kidneys, nephromegaly, collecting system abnormalities, renal cysts, nephrocalcinosis, and medullary sponge kidney [111].

Branchio-oto-renal syndrome (BOR; OMIM #113650) is an autosomal dominant disorder characterized by defects in the derivatives of the branchial arches that give rise to the face, the neck, and the pharynx. BOR syndrome is characterized by cervical fistulas or cysts, ear pits, cochlear hypoplasia, hearing loss, and renal anomalies [85, 112]. The prevalence of BOR syndrome is about 1:40,000 and 2% of affected children have profound deafness. About 40% of patients with BOR syndrome have heterozygous inactivating mutations of the gene that encodes the transcription factor EYA1 on chromosome 8q13 [86]. EYA1 is expressed in nephron progenitor cells (NPC) of the embryonic kidney and in the first branchial arch that gives rise to the external ear, the auditory canal, the ossicles of the ear and the hair cells of the cochlea. Between 5% and 10% of patients with BOR syndrome have mutations in genes belonging to the transcription factor family of SIX proteins. The *EYA1* gene interacts with members of the *SIX* gene family that control expression of the PAX2 and GDNF protein products that are required for specification and/or maintenance of NPCs in the metanephric mesenchyme [113, 114]. Loss of *Six2* in mutant mice results in depletion of the NPCs and renal hypoplasia [115]. To date, one patient with CAKUT has been identified with a pathogenic mutation in *SIX2*: it was not reported if this boy had extrarenal features consistent with BOR syndrome [116]. In BOR syndrome, penetrance is incomplete, and patients may have only one or two features of the syndrome. Renal anomalies are not observed in all patients and may differ between individuals within the same family. Renal findings include renal agenesis or hypoplasia, vesicoureteral reflux (VUR), ureteropelvic junction obstruction (UPJO) and ureteral duplication. Renal hypoplasia ranges from slightly reduced kidney size to bilateral renal agenesis with perinatal death [117]. Only 5–10% of patients with BOR syndrome develop end-stage renal disease.

Campomelic dysplasia (OMIM# 114290) is a rare autosomal dominant condition resulting from a dominant mutation in the *SOX9* gene (*sex-determining protein homeobox 9* mapped to 17q24.3) or from a mutation upstream of the *SOX9* gene [88, 89]. Most cases (77%) are lethal because of respiratory insufficiency from laryngotracheomalacia in combination with a narrowed thorax. Clinical features include hypoplastic scapula and cervical vertebrae, short femur and tibia that are ventrally bowed, dislocated hips, talipes equinovarus (clubfoot), facial abnormalities, including micrognathia and cleft palate (Robin sequence), congenital heart disease, brain defects, and renal abnormalities including hydroureter, renal hypodysplasia, and hydronephrosis [88, 89]. In the developing mouse kidney, SOX9 is expressed in the ureteric bud (UB) tips and the ureteral mesenchyme. Targeted deletion of *Sox9* in the mouse UB results in severe kidney defects ranging from hypoplastic kidneys to renal agenesis [118].

CAKUTHED (OMIM #617641) is an autosomal dominant syndrome of CAKUT with or without hearing loss secondary to mutations in the transcription factor *PBX1* which encodes the *Pre-B cell leukemia transcription factor.* In addition to hearing loss, the outer ear may be malformed and developmental delay is described. Using targeted exome sequencing, Heidet et al. identified five individuals with CAKUT and a de novo pathogenic mutation in *PBX1:* some of these individuals also had deafness and/or developmental delay [87]. *Pbx1* null mice exhibit hypoplastic kidneys, renal agenesis, and poorly defined cortical and medullary regions [119].

DiGeorge syndrome (OMIM #188400) is an autosomal dominant disorder caused by a 1.5–3.0 megabase heterozygous deletion on chromosome 22q11.2. The malformations associated with the deletion include congenital cardiac anomalies, neonatal hypocalcemia from hypoparathyroidism, and thymic hypoplasia. A mild to moderate delay in neurocognitive and psychomotor development is common as well as dysmorphic facial features. Kidney malformations include unilateral renal agenesis, renal dysplasia, and bilateral hydronephrosis [90].

Fraser syndrome (OMIM# 219000, 617666, 617667) is characterized by eyes that are completely covered by skin and usually malformed (cryptophthalmos), fusion of the skin between the fingers and toes (cutaneous syndactyly), and genitourinary anomalies including enlarged clitoris in females, and ambiguous genitalia [91]. The most common urinary tract abnormality in Fraser syndrome is the absence of one or both kidneys. Fraser syndrome results from autosomal recessive mutations in *FRAS1* and *FRAS1-related extracellular matrix* (ECM) *gene 2* (*FREM2*) or *GRIP1*, a scaffolding protein that physically interacts with FRAS1 and FREM2 [120]. Autosomal recessive mutations in the genes implicated in Fraser syndrome are also associated with isolated CAKUT [121]. FRAS1 and FREM2 are extracellular matrix proteins that line the ureteric bud epithelia, Mice lacking *Fras1* develop renal agenesis due to the failure of ureteric bud outgrowth secondary to impaired GDNF expression [122]. The renal phenotype observed in *Fras1* mutant mice can be rescued by decreasing Sprouty activity, which permits FGF-mediated ureteric bud induction [123]. FRAS1 and FREM proteins contribute to epithelial integrity such that mutations in their corresponding genes lead to disrupted dermal-epidermal attachment and embryonic skin blistering [120]. Syndactyly may result from downregulation of MSX2, a transcription factor that stimulates apoptosis and interdigital cell death.

HDR syndrome (hypoparathyroidism, sensorineural deafness and renal disease), also known as Barakat syndrome, is an autosomal dominant form of hypoparathyroidism associated with sensorineural deafness and renal dysplasia (HDR syndrome; OMIM #146255) [92, 93]. HDR syndrome is caused by mutations in the gene encoding GATA binding protein 3 (GATA3), which is localized to chromosome region 10p14–15 [92, 93]. GATA3 is a zinc finger transcription factor that regulates gene expression in many developing tissues such as the parathyroid glands, breast, kidneys, inner ears, and thymus. In the kidney, GATA3 is essential for ureteric bud branching, and mice without *Gata3* fail to develop kidneys. Conditional inactivation of *Gata3* in the nephric duct in mice causes ectopic ureteric budding and a spectrum of urogenital malformations [124]. *Gata3* acts downstream of *β-catenin*, but upstream of *Ret*, to prevent ectopic ureter budding and premature cell differentiation in the nephric duct [124].

Jeune syndrome (also called asphyxiating thoracic dystrophy) (OMIM#611177, 617353) is an autosomal recessive skeletal dysplasia with multiorgan involvement [94]. Some patients have a mutation in the gene, *IFT80* that encodes a protein that is part of the intraflagellar transport complex B, which is critical for the function of sensory and motile cilia [94]. Skeletal abnormalities include short ribs and limbs, narrow thorax, and polydactyly. Patients usually develop progressive respiratory insufficiency in the first few months of life related to the abnormally small thorax. Renal manifestations include cystic kidney disease resulting in renal insufficiency.

Kallman syndrome or idiopathic hypogonadotropic hypogonadism with anosmia (OMIM#300338) is associated with mutations in as many as 25 genes including *ANOS1* or *Anosmin1, FGFR1, FGF8, PROKR2, SPRY4, FGF17, SEMA3A or Semaphorin-3A, IL17RD,* and *FEZF1* [95, 96]. There is marked locus heterogeneity, incomplete penetrance and variable expressivity, and evidence for mutations in multiple genes in some affected families, suggesting oligogenic inheritance. The *ANOS1* gene is located on the X chromosome and encodes the protein anosmin-1 which is found in the extracellular matrix. The clinical phenotype of patients with *ANOS1* mutations is more severe with a higher incidence of microphallus, cryptorchidism, and small testes. The renal phenotype of Kallman syndrome is characterized by unilateral renal agenesis [125]. Semaphorins are secreted guidance proteins that play important roles in organogenesis. In the mouse, SEMA3A regulates vascular patterning in the developing kidney, acting as a negative regulator of endothelial cell migration and survival, and as a negative regulator of ureteric bud branching [126]. Mice with a mutation in *Sema3A* also show abnormal olfactory development [127].

Mammary-ulnar syndrome (OMIM#181450) is an autosomal dominant disorder due to mutations in the *T-box transcription factor 3* (*TBX3*) gene characterized by posterior limb deficiencies or duplications, apocrine/mammary gland hypoplasia, abnormal dentition, delayed puberty in males, genital anomalies, renal dysplasia, and unilateral renal agenesis [97]. Deletion of *Tbx3* in mouse endothelial cells results in glomerular hypoplasia, microaneurysms, and fibrosis in subsets of glomeruli [128].

Mayer-Rokitansky-Kuster-Hauser Syndrome (MRKH, OMIM #277000) is a syndrome that affects 1:5,000 girls. It is characterized by uterovaginal atresia in the setting of a normal female karyotype and is associated with renal agenesis. There is significant phenotypic overlap with another syndrome MURCS which stands for Mullerian duct aplasia, unilateral renal agenesis, and cervicothoracic somite anomalies (OMIM #601076). In a study of 25 patients, Pittock et al. noted that the most common presenting symptom was primary amenorrhea and the mean age of presentation was 16.75 years [98]. Recently, heterozygous mutations in *GREB1L* were identified in fetuses with bilateral renal agenesis and uterovaginal aplasia [66]. The authors observed that mutations in *GREB1L* result in MRKH with incomplete penetrance and therefore, they suggested that there may be a requirement for additional genetic and environmental factors for full penetrance of the phenotype.

Okihiro syndrome, also known as Duane-radial ray syndrome, (OMIM#607323) is an autosomal dominant disorder due to mutations in the *Sal-like 4* (*SALL4*) gene characterized by upper limb, ocular and renal anomalies [99]. Renal anomalies include renal agenesis, crossed fused renal ectopia and VUR [99]. SALL4 is transiently expressed in the nephric duct and ureteric bud during early kidney development in mice.

Pallister-Hall syndrome (PHS, OMIM#146510) is an autosomal dominant disorder characterized by hypothalamic hamartomas, pituitary dysfunction, central polydactyly in which the extra digit is on the ring, middle or index finger, and visceral malformations [100]. Urogenital system defects in individuals with PHS include renal aplasia or hypoplasia, hydroureter, hydronephrosis, or a common urogenital sinus with an imperforate anus [129]. PHS is due to a heterozygous mutation in the *GLI3* gene located on chromosome 7p14 [100]. *GLI3* encodes a zinc finger transcription factor that mediates Sonic hedgehog (SHH) signaling. SHH controls cell fate determination, proliferation and tissue patterning during embryogenesis. Studies in mice demonstrate that urogenital system phenotypes observed in PHS are caused by GLI3R-dependent alterations in nephric duct patterning, ureteric bud branching, and nephron progenitor cell self-renewal [130].

Renal-coloboma syndrome (RCS, OMIM #120330), also known as the papillorenal syndrome, is an autosomal dominant disorder characterized by renal hypoplasia and optic nerve colobomas [62]. Other extrarenal features of RCS include sensorineural hearing loss, Arnold-Chiari malformation, seizures, and joint laxity. Renal malformations include renal hypodysplasia, VUR, and less often, multicystic dysplasia and UPJO [131]. RCS results from mutations in the *Paired box 2* (*PAX2*) gene. PAX2 belongs to a family of PAX DNA-binding proteins that control tissue morphogenesis and embryo patterning. PAX2 has a critical role at multiple steps of kidney and urinary tract development. Most individuals with renal hypoplasia from a mutation in *PAX2* progress to end-stage renal disease (ESRD) [58]. More than 50 heterozygous mutations in the *PAX2* gene have been described in patients with the RCS.

Analysis of genetically modified mouse models has identified renal developmental genes and the gene regulatory networks directing morphogenesis of the urogenital system. In the mouse, *Pax2* null embryos form a nephric duct which later degenerates by apoptosis during the elongation process and thus, they do not form normal mesonephric tubules [132]. As a result, *Pax2*-deficient mouse embryos fail to form metanephric kidneys. Heterozygous inactivation of *Pax2* in the mouse leads to renal hypoplasia and vesicoureteral reflux [133]. The $Pax2^{1\mathrm{Neu}/+}$ mouse model, bearing the same 1-bp frame-shift mutation in *Pax2* as found in a human family with RCS, recapitulates the defects of RCS, including renal hypoplasia, cystic kidneys, and VUR [133]. PAX2 can switch from a positive to a negative regulator of gene transcription through interaction with the co-repressor protein Groucho-related gene 4 (GRG4). In addition, PAX2 can regulate gene transcription through its interactions with Ptip (Pax transactivation domain interacting protein) by recruiting epigenetic complexes to the DNA [134]. Phosphorylated PAX2 recruits Ptip and a complex of H3K4 methyltransferases including the mammalian homologues of Drosophila Trithorax proteins known as MLL (mixed lineage leukemia) that promote chromatin opening and active gene transcription via H3K4 trimethylation

[134]. The downstream targets of PAX2 include genes critical for normal kidney morphogenesis. In the nephric duct, PAX2 regulates the transcription of *GATA3* and *LHX1* which turn on the GDNF (glial derived neurotrophic factor)/RET pathway which is necessary for metanephric kidney induction and UB branching [124, 135]. Later in kidney development, PAX2 has a distinct role in mesenchymal-epithelial transition, specification, and patterning of the surrounding mesenchyme. Based on the analyses of *Pax2* loss of function phenotypes in the mouse, *PAX2* is critical for the development of both the ureteric bud and the metanephric mesenchyme.

Renal cysts and diabetes (RCAD) syndrome (OMIM #137920) is an autosomal dominant disorder caused by mutations of the *TCF2* gene (also called *HNF1β* or *HNF1 Homeobox B*) that encodes hepatocyte nuclear factor-1-β (HNF-1-β) protein [101]. HNF-1-beta protein is a member of the homeodomain-containing superfamily of transcription factors and regulates gene expression in several organs, including the liver, the kidney, the intestine, and the pancreas. Clinical features of RCAD syndrome include renal cysts, diabetes mellitus, elevated liver enzymes, hyperuricemia, pancreatic atrophy/hypoplasia, and genital malformations [136]. There is no correlation between the genotype and the phenotype and there is a high intrafamilial variability of the phenotype. De novo mutations are discovered in 50% of cases [136]. In case series of children with renal cysts, renal hypodysplasia or syndromic CAKUT, mutations of *HNF1β* have been identified in 15–30% of cases [137]. Mutations in *HNF1β* account for 29% of fetuses with hyperechogenic kidneys of normal size or slightly enlarged size and renal cysts are also often observed [25]. In the Decramer study, the most prevalent genetic anomaly was the complete heterozygous deletion of the *HNF1β* gene, which was observed in 83% of cases [25]. The mechanisms by which HNF1β might be involved in renal cystogenesis in humans are unknown. Studies in animal models suggest that HNF1β may control renal cyst formation by regulating genes that are critical for kidney development and ciliogenesis. Targeted removal of *Hnf1β* in mice leads to abnormal ureteric bud branching and defective collecting duct differentiation/patterning which leads to cystogenesis [138]. At a molecular level, *Hnf1β*-deficient mice exhibit reduced expression of key regulators of ureteric bud branching, such as PAX2, RET, GFRA1 and ETV5 [138]. Other animal studies demonstrate that mice with renal-specific inactivation of *Hnf1β* develop polycystic kidney disease. HNF-1β may promote renal cyst formation by regulating the transcription of genes involved in cystogenesis such as *UMOD*, *PKHD1* (polyductin), *PKD2*, and *Polaris* [139]. These genes play a critical role in the formation of cilia.

Renal tubular dysgenesis Homozygous or compound heterozygous mutations in the genes encoding for angiotensinogen (*AGT*), renin, angiotensin-converting enzyme (*ACE*) or angiotensin II receptor type 1 (*AGTR1*) are associated with renal tubular dysgenesis (RTD, OMIM #267430) [102]. The critical role of the renin-angiotensin system (RAS) during kidney development in humans was revealed by the fact that maternal use of ACE inhibitors or ATR1 antagonists during pregnancy causes fetal anuria, oligohydramnios, and low blood pressure in neonates postnatally. All the major components of the RAS are expressed in the human embryonic kidney as early as the fifth week of gestation when metanephric organogenesis commences. To date, 54 distinct mutations have been identified in 48 unrelated families [102, 140]. These mutations appear to be pathogenic because they result in the absence of protein or they modify highly conserved amino acid residues [140]. Affected patients exhibit severe hypotension, which is presumably due to the lack of angiotensin II. Most newborns with RTD die in the perinatal period due to anuria combined with pulmonary hypoplasia secondary to the severe oligohydramnios [141]. Skull ossification defects are frequently observed and result in microcephaly, large fontanelles, and underdeveloped cranial bones. The kidneys are usually of normal size. Glomerular number may be normal, low, or increased, while glomerular size is normal or enlarged. The hallmark of the disease is the absence or the reduction in proximal tubules. Those proximal tubules that are observed are immature and lack a brush border. Typical vascular changes include thickening of the muscular layer of small arteries and arterioles. In the medulla, collapsed CDs and abundant interstitial fibrosis are observed [142]. No correlation has been established between the mutated gene and the clinical course of the disease, suggesting that the disease is not specifically linked to the defect of one or another *RAS* gene, but to low systemic blood pressure, which is the physiological consequence of RAS inactivation.

A single polymorphism, a 1332A>G transition, in the human *AGTR2* gene, that encodes angiotensin II receptor type 2 has been associated with CAKUT in several, but not all, studies. This alteration in the *AGTR2* gene is described in American and German Caucasian males with ureteropelvic junction (UPJ) stenosis, megaureter, and multicystic dysplastic kidney (MCDK) [143]. The same transition was detected in Korean children with unilateral MCDK, renal agenesis, and hydronephrosis [144], and in Italian children with VUR, hypoplastic kidney, UPJ stenosis, nonobstructive megaureter, and posterior urethral valves (PUV) [141]. The difference in phenotypes may be due to an insufficient number of patients studied, limitations in study design, low penetrance of *AGTR2* mutation, the contribution of modifier genes, or other factors. Thus, while *RAS* gene mutations have been clearly linked to patients with RTD, their association with other types of CAKUT in humans remains elusive.

Rubinstein-Taybi syndrome (RTS, OMIM #180849) is an autosomal dominant disease due to a contiguous deletion of chromosome 16 p13.3 that includes the cAMP response element-binding protein (CREB)-binding protein gene, *CREBBP* (also called *CBP*) as well as neighboring genes. Less severe forms of the syndrome are due to point mutations or deletions restricted to the *CBP* gene and account for 50–70% of affected patients. Another gene, *EP300* on chromosome 22q13 accounts for 3% of affected patients [145]. The *EP300* gene encodes the protein p300, which regulates the transcription of many genes and is important in cell proliferation and cell differentiation. Both CBP and p300 are histone acetyltransferase enzymes that remodel chromatin such that transcription factor proteins can bind to genomic DNA. RTS is characterized by short stature, microcephaly, dysmorphic features, broad thumbs and toes, hirsutism, congenital heart disease and intellectual disability [103]. Renal anomalies consist of renal hypoplasia and agenesis. Homozygous removal of *Cbp* or *p300* in the mouse leads to an embryonic lethal phenotype prior to kidney formation. The cAMP pathway has been shown to be important for the release of renin and the differentiation of renin-producing cells. Indeed, conditional deletion of *Cbp* and *p300* in renin-expressing cells in mice results in severe kidney abnormalities which include an absence of renin-producing cells and structural abnormalities with a disorganized corticomedullary junction, and cortical and medullary cysts. This demonstrates that CBP and p300 are important for kidney development [146].

Simpson-Golabi-Behmel syndrome (SGBS; OMIM#312870) is an X-linked disorder in which patients display overgrowth, a distinct facial appearance, macroglossia, cardiac defects, dysplastic and cystic kidneys, hernias, supernumerary nipples, and various skeletal abnormalities, including polydactyly and syndactyly [147]. SGBS is due to mutations in the *glypican-3* (*GPC3*) gene which encodes a cell-surface glycoprotein [104]. Glypicans are a family of heparan sulfate proteoglycans that are linked to the cell surface through a glycosyl–phosphatidylinositol anchor. *Gpc3*-deficient mice reproduce clinical features observed in SGBS patients, including developmental overgrowth, perinatal death, cystic and dysplastic kidneys, and abnormal lung development [148]. Analysis of *Gpc3*-deficient mice has revealed that there is increased proliferation of the ureteric bud cells early in development and this is followed by selective degeneration of medullary collecting ducts that results in renal cystic dysplasia. Mechanistically, GPC3 inhibits renal branching morphogenesis by modulating BMP2-SMAD signaling [149].

Smith-Lemli-Opitz syndrome (SLOS; OMIM #270400) is an autosomal recessive syndrome caused by an inborn error of cholesterol metabolism from a mutation in the *DHCR7* gene on chromosome 11q13. The gene *DHCR7* encodes the enzyme 7-dehydrocholesterol reductase (7-DHC) that catalyzes the conversion of 7-DHC to cholesterol [105]. Metabolically, SLOS is characterized by reduced serum cholesterol levels and phenotypically by cognitive disability, facial dysmorphisms, syndactyly of the second and third toes, photosensitivity and holoprosencephaly in severe cases. Mild cases exhibit minimal physical abnormalities and near-normal intelligence [105]. Renal features include renal agenesis and dysplasia [105]. The molecular mechanisms leading to the renal developmental malformations found in SLOS patients are unknown, however, kidneys from *Dhcr7*$^{-/-}$ mice exhibit a defect in UB branching [150].

Townes-Brocks syndrome (TBS; OMIM #107480) is an autosomal dominant disorder caused by mutations in the *SALL1* gene. The *SALL1* gene is located on chromosome 16q12.1 and encodes a zinc finger transcription factor which is required for kidney development [106]. The syndrome is characterized by external ear anomalies, imperforate anus, preaxial polydactyly with triphalangeal thumbs, and cardiac anomalies [151]. The renal malformations reported in TBS consist of hypoplastic and/or dysplastic kidneys, renal agenesis, horseshoe kidney, multicystic dysplastic kidney, VUR, and PUV [106]. TBS occurs in 1 in 200,000 live births. De novo mutations in *SALL1* are observed in 50% of cases. Approximately 65% of patients have point mutations, and 5% deletions. The severity of the disease varies and is not correlated with the genotype. Mice deficient in *Sall1* die in the perinatal period with kidney agenesis [152]. *Sall1* is expressed in the metanephric mesenchyme surrounding the ureteric bud. Homozygous deletion of *Sall1* results in apoptosis of the adjacent metanephric mesenchyme and incomplete ureteric bud outgrowth which highlights the importance of the reciprocal signaling between these tissues for the formation of the metanephric kidney [152]. In addition, SALL1 is important for self-renewal and differentiation of nephron progenitor cells that are controlled, in part, by the transcription factor SIX2. Conditional deletion of *Sall1* in Six2-positive nephron progenitor cells results in severe depletion of this stem cell population and a marked reduction in nephron formation in the mouse. SALL1 regulates the balance between nephron progenitor cell self-renewal and differentiation by activating progenitor-related genes in SIX2-positive nephron progenitors and by repressing gene expression in SIX2-negative differentiating nascent nephrons [153].

Zellweger syndrome (ZWS; OMIM #214100), also known as cerebrohepatorenal syndrome, is an autosomal recessive disorder due to mutations in the pexin genes including *PEX1*, *PEX2*, *PEX3*, *PEX5*, *PEX6*, *PEX10*, *PEX12*, *PEX13*, *PEX16*, *PEX19*, or *PEX26* that are involved in peroxisome biogenesis. *PEX* genes are required to import protein and fatty acids from the cytosol into peroxisomes with subsequent enzymatic degradation. ZWS is characterized by craniofacial dysmorphisms (high forehead, widely separated cranial sutures, epicanthal folds, upward slant of the eyes), cataracts, pigmentary retinopathy, hepatomegaly with biliary dysgenesis, developmental delay, hypotonia and seizures [107]. Renal anomalies include glomerulocystic kidney disease and VUR [107].

Trisomies and CAKUT

Trisomy 13, 18, and 21 are relatively common causes of syndromic CAKUT. Trisomy 21 occurs in 1.5 in 1,000 births, while Trisomy 13 and 18 occur in 0.05 and 0.15 per 1,000 live births, respectively [154]. Approximately 5% of individuals with Trisomy 21 have CAKUT [155]. The most common forms of CAKUT in children with trisomies 13, 18, and 21 include hydronephrosis, horseshoe kidney, renal hypoplasia, duplex kidney/collecting system, cortical cysts, and cystic dysplasia. The mechanisms by which trisomies may cause CAKUT are unknown. Characteristic clinical features of Trisomy 21 (Down syndrome) include epicanthic folds, intellectual disability, congenital heart disease (atrioventricular septal defects, Tetralogy of Fallot), duodenal atresia, short stature, hypospadias, and cryptorchidism [156]. Trisomy 13 (Patau syndrome) is characterized by holoprosencephaly, microphthalmus, cleft lip and/or palate, congenital heart disease, and polydactyly. The major phenotypic features of Trisomy 18 (Edwards syndrome) include IUGR, hypertonia, congenital heart disease (ventricular septal defects and patent duct arteriosus), Meckel's diverticulum, and intestinal malrotation [157].

Environmental Factors that Predispose to Congenital Anomalies of the Kidney

From questionnaire data of 562 parents of children with CAKUT, maternal obesity and maternal diabetes were shown to be risk factors for CAKUT. Interestingly, maternal obesity was highly associated with duplex kidneys and VUR, while maternal diabetes was especially associated with posterior urethral valves [158]. Dart et al. demonstrated that pregestational diabetes was significantly associated with CAKUT (odds ratio, 1.67; 95% confidence interval, 1.14–2.46), which implies a 67% increased chance of CAKUT in the offspring of mothers with pregestational diabetes compared to the general population (8.3 vs. 5.0 per 1,000 births, respectively) [159]. In rat studies, maternal hyperglycemia and diabetes mellitus have also been linked to CAKUT [160].

Evidence derived from human and animal model studies demonstrates an important role for the intrauterine environment in the pathogenesis of CAKUT [161] (Table 5). Intrauterine growth restriction (IUGR), defined as fetal weight below the 10th percentile for gestational age, or low birth weight (<2500 g) strongly suggest that the in-utero environment is suboptimal. Maternal undernutrition is one of the most common causes of human IUGR [177]. In a variety of animal models, maternal dietary protein restriction results in decreased nephron number with impaired kidney function and hypertension [169]. Maternal protein restriction during pregnancy results in increased apoptosis of nephron progenitor cells and it alters gene expression in the developing kidney [170]. One specific mechanism by which maternal low protein diet may cause renal hypoplasia is by increasing fetal exposure to maternal glucocorticoids by downregulating placental production of the enzyme 11β-hydroxysteroid dehydrogenase type 2 which converts physiological glucocorticoids to inactive products. In animal models, high maternal glucocorticoid levels impair nephron formation in the fetal kidney. A maternal diet that is low in protein can also inhibit

Table 5 In utero environmental factors associated with CAKUT

Fetal exposure to	Renal phenotype	Reference(s)
ACE inhibitors or ARBs	Renal tubular dysgenesis (absence or poor differentiation of proximal tubules, retraction of glomerular tufts, thickening of arterial walls)	[162, 163]
Alcohol	Agenesis, ectopia/horseshoe, cystic dysplasia, hypoplasia, hydronephrosis/ureter	[164, 165]
Cocaine	Agenesis, hypoplasia, hydronephrosis/ureter	[166]
Dexamethasone/ Corticosterone	Hypoplasia	[167, 168]
Hyperglycemia	Agenesis, ectopic/horseshoe kidney, cystic/dysplasia, hypoplasia, hydronephrosis/ureter	[158, 159, 160]
Low protein diet	Hypoplasia	[169, 170]
Maternal obesity	Duplex kidneys, VUR	[158]
Thalidomide	Agenesis, ectopic/horseshoe kidney, cystic dysplasia, hypoplasia, hydronephrosis/ureter	[171, 172]
Uteroplacental insufficiency	Hypoplasia	[173]
Valproic acid	Hydronephrosis, ectopic kidney, MCDK	[174]
Vitamin A deficiency	Hypoplasia, hydronephrosis/ureter	[175, 176]

ACE Angiotensin-converting enzyme, *ARB* Angiotensin II AT1 receptor blocker fetal activation of the renin-angiotensinogen-system which results in impaired nephrogenesis [178–180]. Interestingly, when pregnant dams on a low protein diet receive retinoic acid, a metabolite of Vitamin A, kidney size and nephron number is normalized in the offspring [181]. Similarly, pregnant dams that are fed a Vitamin A deficient diet give birth to offspring with renal hypoplasia, a decrease in glomerular number, hydronephrosis and hydroureter [175, 176]. Indeed, retinoic acid is a potent morphogen during embryogenesis that signals through the retinoic acid receptors (RAR) to regulate apoptosis, differentiation, and cell fate specification. Deletion of *Rarα* and *Rarβ2* in mice causes renal hypoplasia and reduced expression of *Ret* in the ureteric bud [182].

Maternal uteroplacental insufficiency is another important cause of IUGR with secondary low nephron endowment in the fetus and is seen in women with hypertension, pre-eclampsia, and diabetes. The main cause of maternal uteroplacental insufficiency is vascular in which the spiral arteries that nourish the fetus fail to become low resistance, high flow vessels. Maternal uteroplacental insufficiency induced by incomplete bilateral uterine vessel ligation in animal models also has direct effects on fetal kidney development. In the rat model, uterine vessel ligation results in a decrease in glomerular number and renal hypoplasia in the offspring [173].

Several other environmental factors are risk factors for impaired nephrogenesis and CAKUT. In utero exposure to several medications, illicit drugs and alcohol have all been associated with renal dysplasia. Maternal use of renin-angiotensin system inhibitors such as ACE inhibitors, Angiotensin II receptor blockers, and direct renin inhibitors during pregnancy have been linked to an increased risk of fetopathy in humans. Most children manifest hyperechogenic and enlarged kidneys with proximal tubular dysplasia, thickening of arterial walls and multiple small cysts [162, 163].

Folic acid is required for cell division and cell maintenance and is an essential factor in DNA methylation, which is critical for epigenetic regulation of gene expression. Maternal supplementation with folate has been shown to decrease the risk of a neural tube defect in the developing fetus and there may also be benefit in prevention of CAKUT. Folic acid may reduce the incidence of urinary tract malformations [183]. Importantly, CAKUT phenotypes have been observed in infants exposed to folic acid antagonists in utero, such as carbamazepine, phenytoin, primidone, phenobarbital, or valproic acid, suggesting that folate is important for kidney and urinary tract development [174, 183].

Dexamethasone, a synthetic glucocorticoid that readily crosses the placental barrier, has also been shown to be detrimental to fetal kidney development. Animal studies have demonstrated that fetal dexamethasone exposure is linked to a reduction in nephron endowment and renal hypoplasia [167, 168]. Thalidomide is a drug with immunomodulatory and antiangiogenic characteristics that was originally prescribed for morning sickness and now is used to treat some cancers and skin conditions. Thalidomide use during pregnancy is teratogenic and induces severe limb deformities or phocomelia as well as CAKUT and renal hypoplasia [171, 172].

Maternal use of illicit drugs and alcohol are also risk factors for CAKUT. Children exposed to cocaine in utero have an increased risk of renal agenesis and hypoplasia [166]. While the mechanism is not clear, this could be due to cocaine-induced arterial vasoconstriction with uteroplacental insufficiency or maternal anorexia. Maternal alcohol consumption may cause Fetal Alcohol Syndrome which includes renal system anomalies [164, 165]. Here again, the mechanism is not known, but it may be secondary to the effect of alcohol on Vitamin A metabolism: retinol is metabolized to retinoic acid via alcohol dehydrogenase. Alcohol dehydrogenase may be inhibited in the setting of elevated levels of ethanol resulting in a deficiency of retinoic acid [184].

In summary, the underlying molecular mechanisms that cause CAKUT in response to a suboptimal in utero environment remain to be elucidated. However, there is strong animal data and some human data that reveals the importance of the in-utero environment for fetal development including the kidneys and urinary tracts. Further research in this area is essential to improve early diagnosis, prevention, and treatment of CAKUT and its associated comorbidities.

Cross-References

- ▶ Imaging Kidneys and the Urinary Tract
- ▶ Pediatric Obstructive Uropathy
- ▶ Pre-natal Development of the Kidneys and Urinary Tract
- ▶ Vesicoureteral Reflux in Children

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Stem Cells and Kidney Regeneration

4

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Abstracts

Because of the few effective treatments for many chronic kidney diseases, increasing consideration has been given to new regenerative therapies using stem cells. Recent advances in developmental biology and stem cell biology have led to the increased availability of renal stem/progenitor cells from multiple sources,

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including their isolation from embryonic and adult kidneys, and to the generation of kidney lineage cells by the directed differentiation of pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), or by the cellular reprogramming of fully differentiated adult renal cells or across mature lineages. Cell therapies using renal stem/progenitor cells derived from adult and embryonic kidneys or differentiated from ESCs/iPSCs have been investigated in kidney disease models. The reconstruction of transplantable kidney organs is also being attempted using ESC/iPSC-derived kidney lineage cells. Cancer stem cells, which are thought to initiate and maintain malignant tumors, have been identified in renal cell carcinomas (RCC) and in Wilms' tumor (WT), the most common pediatric renal malignancy. The development of regenerative treatment using stem cells and their bioproducts and targeted therapies against renal cancer stem cells may open new avenues in renal medicine.

Keywords

Renal stem cell · Renal progenitor cell · Kidney development · Kidney regeneration · ESC · iPSC · Cellular reprogramming · Niche · Cancer stem cell · Wilms' tumor

Introduction

Any pathologic process that leads to the loss of nephrons, which are the functional units of the kidney, may eventually cause decreased renal function, a process collectively termed chronic kidney disease (CKD) [1]. CKD gradually progresses to end-stage kidney disease (ESKD), and patients with ESKD only have two therapeutic options: renal transplantation and dialysis therapy. Renal transplantation is hampered by serious problems, including the shortage of donor organs, chronic use of immunosuppressive drugs, and chronic allograft failure. Dialysis therapy can substitute for a part of renal function, but eventually causes systemic complications. The lifespan of patients with renal transplants and on dialysis is also typically shortened compared to the general population for many reasons including cardiac disease, infections, neoplasia, etc. Also, the prevalence of CKD is increasing worldwide, which gives growing economic burden to health-care systems [1]. Since CKD represents a gradual loss of nephrons, substantial efforts have been made towards kidney regeneration using stem cells.

Stem cells have self-renewal capacity and differentiation potential into multiple cell types constituting the body. Progenitor cells that differentiate from stem cells are more developmentally committed to specific cell lineages. These two cell types are sometimes difficult to clearly distinguish and often equated. Stem cells are largely classified into two categories: tissue-specific or somatic stem cells and pluripotent stem cells (PSCs). Tissue-specific stem cells have been identified in most organs or tissues, including the kidney. They generally show relatively limited proliferation capability on culture dishes and limited differentiation potential into a restricted number of cell types constituting the original organ or tissue. In contrast, PSCs, such as embryonic stem cells (ESCs) [2] and induced pluripotent stem cells (iPSCs) [3, 4], have virtually unlimited replicative capacity and are theoretically able to give rise to any cell type in the body. Like other clinically useful cell types, vigorous efforts to differentiate kidney lineage cells from PSCs have been undertaken [5, 6].

An increasing number of reports have described the successful generation of desired cell types by manipulating core transcriptional factors to convert fully differentiated cells to a pluripotent state or from one fully differentiated cell type to another. This experimental strategy, termed cellular reprogramming, has been utilized to generate kidney lineage cells [5, 6].

In parallel to the above, a growing body of evidence has supported the idea that malignant tumors are initiated and maintained by cancer stem cells (CSCs), which share similar biologic properties with normal stem cells, showing the ability to self-renew as well as give rise to differentiated tissue cells [7, 8]. While most knowledge on CSCs had initially came from leukemia stem cells, cancer stem cell populations have also been identified from solid tumors, including adult and pediatric renal cancers [9]. The elucidation of the biological properties of CSCs would allow the development of therapies that target these malignant stem cell populations.

This chapter will summarize the current status of research on kidney regeneration using renal stem/progenitor cells isolated from embryonic or adult kidneys and kidney lineage cells generated by the directed differentiation of PSCs or by the cellular reprogramming of other cell types. Present knowledge on CSCs of kidney-related malignancies will also be described.

Embryonic Renal Stem/Progenitor Cells

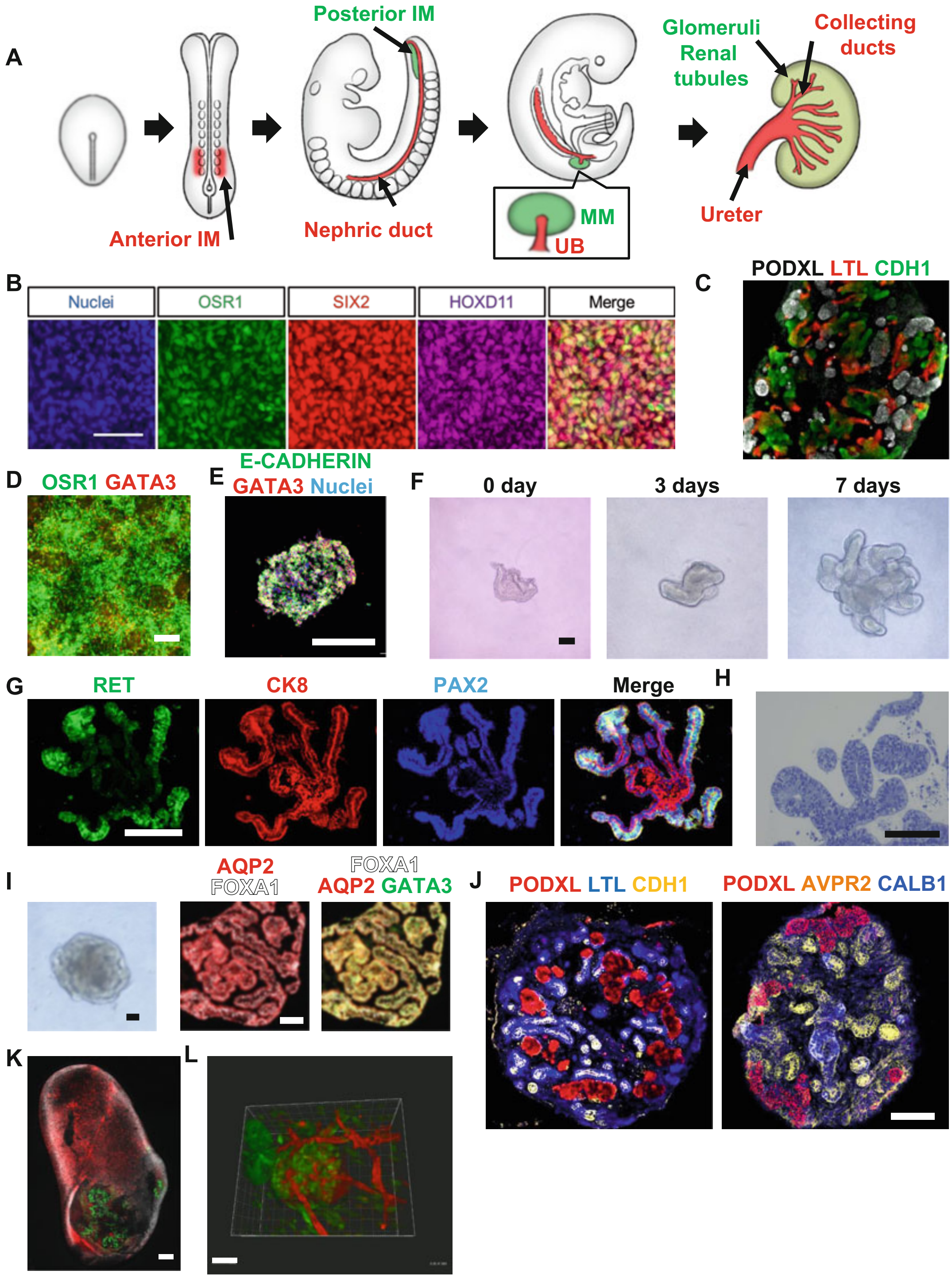
Kidney Development

To understand the entity of embryonic renal stem/progenitor cells, this section starts by briefly summarizing mammalian kidney development (Fig. 1a; [10, 11]) (see ▶ Chap. 1, "Pre-natal Development of the Kidneys and Urinary Tract"). The kidneys are derived from an early embryonic germ layer, the intermediate mesoderm (IM; [12]), which is located between the lateral and paraxial mesoderm and derived from the primitive streak, which is the progenitor population for both the mesoderm and endoderm. A number of factors, such as WNT, bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and Nodal/activin and retinoic acid (RA) signals, contribute to the cell fate commitment of primitive streak or mesodermal lineage, including the IM. Especially, BMP signaling is the key morphogen regulating lateral-medial patterning of early mesoderm development. Low BMP induces IM, while high BMP induces lateral plate mesoderm, and the antagonism of BMP signals is required for paraxial mesoderm differentiation [13].

In vertebrates, the IM successively develops three types of kidneys: the pronephros, mesonephros, and metanephros. The three kidneys are similar in that they consist of a basic functional unit, the nephron, although the number of nephrons differs among the three. Mesonephros is the adult kidney of fish and amphibians, while metanephros is the functional kidney of adult amniotes, reptiles, birds, and mammals. The mammalian adult kidney, the metanephros, is formed by reciprocally inductive interactions between two embryonic precursor tissues derived from the IM: the metanephric mesenchyme (MM) and the ureteric bud (UB), a derivative of the nephric (Wolffian) duct. The UB induces the MM to differentiate into epithelialized nephrons and interstitium, while the MM directs the UB to elaborate the lower urinary tract from the collecting ducts through the renal pelvis and ureter to a part of the urinary bladder [14]. Taguchi et al. redefined the origin of the MM and elucidated the lineage commitment from the nascent mesoderm through the IM into either the MM or UB [15]. They demonstrated that the MM is generated from the posterior IM, which is distinct from the anterior UB precursor IM population.

A portion of MM cells condenses around the tips of the UB and differentiates into structures termed cap mesenchyme (CM). CM cells self-renew at the UB tips and give rise to various cell types comprising the nephron. Thus, the CM has been considered multipotent nephron stem/progenitor cells, which can self-renew and differentiate into different types of nephron epithelia [16]. Additionally, CM cells express a unique combination of transcription factors, such as the *Hox11* paralogs, *Osr1*, *Pax2*, *Eya1*, *Wt1*, *Six2*, *Sall1,* and *Cited1*, before UB induction.

The MM produces the secreted neurotrophin, glial cell line–derived neurotrophic factor (GDNF; [17]), which is a chemotactic factor for UB. GDNF drives the outgrowth of the UB from within the nephric duct and the extension of the UB toward the MM. The attracted UB in turn secretes WNT9b, which is the primary inducer for the MM [18] to induce the adjacent MM to condense around the UB tip, thereby forming the CM. Thereafter, WNT4 secreted from the induced CM epithelializes the CM itself in an autocrine fashion [19]. During the process of mesenchymal-epithelial transition (MET), the CM cells sequentially go through specific developmental stages including pre-tubular aggregates, renal vesicles,


A
Posterior IM
Glomeruli
Renal
tubules
Collecting
ducts
MM
UB
Ureter
Anterior IM
Nephric duct
B
Nuclei
OSR1
SIX2
HOXD11
Merge
C
PODXL LTL CDH1
D
OSR1 GATA3
E
E-CADHERIN
GATA3 Nuclei
F
0 day
3 days
7 days
G
RET
CK8
PAX2
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H
I
AQP2
FOXA1
FOXA1
AQP2 GATA3
J
PODXL LTL CDH1
PODXL AVPR2 CALB1
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Fig. 1 Directed differentiation of human PSCs into kidney lineages. (**a**) A schematic showing mammalian kidney development. (**b**) Immunostaining for OSR1, SIX2, and HOXD11in nephron progenitor cells (NPCs) differentiated from human iPSCs. (**c**) Triple immunostaining for PODOCALYXIN (PODXL; podocyte marker; white), *Lotus tetragonolobus* lectin (LTL; proximal tubule marker; red), and CADHERIN 1 (CDH1; distal tubule marker; green) in a nephron organoid formed from human iPSC-derived NPCs after 10 days of air-liquid interface culture.

and C- and S-shaped bodies, and then eventually differentiate into all nephron segments ranging from glomerular podocytes to distal renal tubules.

Among the key molecules involved in the process of kidney development, a homeodomain transcriptional factor, *Six2*, which serves as the most specific marker of CM cells, has been shown to play a central role in the regulation of renal progenitor CM cells, particularly on the ability of CM cells to continuously self-renew throughout nephrogenesis [16]. It was reported that FGF9 and FGF20 are required for the formation and maintenance of the CM progenitors, respectively [20]. MM survival in vitro also depends on FGF2 and BMP7 [21].

Upon completion of the kidney development, transcription factors expressed in the CM, including *Six2*, are no longer expressed and the embryonic nephron progenitors disappear, which leads to no formation of new nephrons. Embryonic renal progenitors do not exist in adult mice even after acute kidney injury (AKI) in ischemia-reperfusion (I/R) models, which were elegantly demonstrated by a lineage tracing study for *Six2* [22]. In contrast to mammals, which lose the renal progenitor pool around birth, a self-renewing nephron stem cell population has been shown to exist in the adult kidneys of lower organisms, such as zebrafish and drosophila, and to form new nephrons throughout adult life, contributing to regenerating nephrons de novo after injury [23, 24].

In addition to the *Six2*-expressing CM progenitors that give rise to several epithelial cell types constituting nephrons, such as glomerular podocytes and renal tubular epithelia, the developing kidney also contains progenitor cell types of other lineages. These include FOXD1(+) stromal progenitors in the MM, which differentiate into renal interstitial cells, mesangial cells, and vascular smooth muscle cells and pericytes in adult kidney [25–28]. Kobayashi et al. performed lineage tracing experiments and showed that the *Foxd1*-expressing cortical stroma represents a distinct multipotent self-renewing progenitor population that gives rise to the adult renal cell types described above throughout kidney organogenesis [28]. Interestingly, it was demonstrated that a small subset of FOXD1(+) stromal cells contributes to SIX2(+) nephron progenitors at early developmental stages, but thereafter a strict nephron and stromal lineage boundary is formed between the nephron and stromal progenitor compartments.

Most knowledge on kidney development has been obtained from genetic approaches with experimental animal models, including fish, frog, chick, and mice [5, 6, 10, 11]. Previous work has also examined embryonic kidneys in human and nonhuman primates, such as rhesus monkey. Humans, nonhuman primates, and mice share several molecules and corresponding spatial expression patterns that act as developmental markers, although major

Fig. 1 (continued) (**d, e**) Immunostaining of anterior intermediate mesoderm (IM) cells for OSR1 (green) and GATA3 (red; **d**) and a nephric duct cell aggregate for E-CADHERIN (green), GATA3 (red), and nuclei (blue; **e**). (**f**) Morphological change during the reconstruction of branching UB organoids for 7 days. (**g**) Immunostaining of a UB organoid for RET (green), CK8 (red), and PAX2 (blue). (**h**) Toluidine blue staining of a UB organoid showing tubular lumens. (**i**) Bright-field (left panel) and immunostaining images (middle and right panels) of collecting duct-like tubular structures derived from a UB organoid for FOXA1 (white), AQP2 (red), and GATA3 (green). (**j**) Triple immunostaining images of human iPSC-derived kidney organoids for markers of podocytes (PODXL), proximal tubules (LTL), and distal tubules and collecting ducts (CDH1; left panel) and for PODXL and markers of distal tubules and collecting ducts (AVPR2) and collecting ducts only (CALB1; right panel). Note that weak CDH1 signals were also found in parts of the LTL(+) proximal tubules in the left panel. (**k**) A low magnification image showing the host kidney and human iPSC-derived kidney graft (green) after the administration of rhodamine B-conjugated dextran through the tail vein of the host mouse. (**l**) Intravital imaging with multiphoton microscopy after the tail vein injection of rhodamine B-conjugated dextran shows the vessel lumens of host mice penetrating into the human iPSC-derived glomerulus structure (green). Scale bars, 100 μm in (**b**)–(**j**), 500 μm in (**k**) and 40 μm in (**l**). ((**b**), (**c**), (**j**)–(**l**) are adapted from Tsujimoto et al. [13], (**d**) and (**e**)–(**i**) are adapted from Mae et al. [57, 58], respectively) differences have been found in the temporal patterns of kidney development. Nephrogenesis starts in human and nonhuman primates at the end of the first trimester and continues throughout the mid-third trimester with a term of 165 ± 10 days, while mouse nephrogenesis begins in mid-gestation and completes postnatally with a term of 20 days [5, 6, 10, 11].

Identification of Embryonic Renal Stem/Progenitor Cells

As described above, the metanephric kidney contains several stem/progenitor cell populations required for the formation of a fully functional kidney. This property has also been demonstrated by the transplantation of whole embryonic kidney rudiments into extrarenal space of host animals. Hammerman et al. [29] and Dekel et al. [30, 31] demonstrated the formation of vascularized renal tissues from transplanted metanephroi inside the rodent body. Notably, when mouse, rat, porcine, or even human early-gestational metanephroi are transplanted into the kidney subcapsular space or the abdominal cavity of immunodeficient mice, they survive, grow, undergo complete nephrogenesis, and form functional organs that are able to produce dilute urine by integrating with the host vasculature and forming vascularized glomeruli comprised of donor-host connections [31]. These seminal studies paved the way for transplantation studies using fetal-like organoid kidney tissue derived from PSCs to achieve host-derived vascularization and maturation [32].

In addition to the transplantation of whole metanephric rudiments, Kim et al. reported that transplantation of a heterogeneous population of dissociated embryonic day (E) 14.5 and E17.5 rat fetal kidney cells under the renal capsule of a 5/6 nephrectomy kidney injury model resulted in the formation of renal structures and improved renal functions [33].

The selection or isolation of specific lineage of progenitor cells included in embryonic kidneys have also been performed either by sorting based on the expression of specific markers or by selective in vitro assays. By creating a clonogenic assay system using *Wnt4*, an epithelializing factor for MM in embryonic development, Osafune et al. demonstrated that the MM contains multipotent nephron progenitor cells (NPCs) that can give rise to several kinds of epithelial cells, such as glomerular podocytes and the epithelia of the proximal and distal renal tubules and Henle's loop [34]. The progenitor cells are contained only in the cell population that strongly expresses *Sall1*, a zinc finger transcription factor that is essential for kidney development and can reconstitute three-dimensional (3D) kidney structures in an organ culture setting that contain glomeruli- and tubule-like components. This clonogenic assay, however, has not yet been applied to human fetal kidney and cannot be used to expand undifferentiated NPCs.

Other approaches to isolate progenitor cells include culturing them as spheres based on the ability of progenitor cells to grow in low-attachment conditions. Lusis et al. established nephrospheres from murine embryonic kidneys, which robustly propagated in vitro, but displayed limited nephrogenic capacity [35]. Recently, several groups have developed in vitro expansion culture methods for mouse and human embryonic NPCs [36–38]. In particular, Li et al. expanded SIX2(+) mouse NPCs over 17 months with more than 110 passages and NGFR(+)EPCAM(+) human NPCs from gestational week (GW)11 human fetal kidneys more than 7 months with 50 passages by using combinations of growth factors and chemical compounds and 3D floating cell aggregation cultures [38].

Methods to prospectively isolate fetal kidney progenitor cells using surrogate cell surface markers of intracellular renal transcription factor expression have also been used to potentially supply an unlimited source of nephron progenitors; these in turn could be used to regenerate damaged epithelial cells within the nephron. Using a previously reported isolation method for the putative progenitor population in adult kidney, Lazzeri et al. isolated a similar CD24(+)CD133(+) cell population from human fetal kidneys [39]. They reported that the CD24(+)CD133(+) population represents 35–50% of early embryonic kidney cells, but gradually decreases in proportion and becomes restricted to the urinary pole of the Bowman's capsule with age. This population may possibly persist into adulthood as progenitors. Importantly, when isolated and injected into immunodeficient mice with acute kidney injury (AKI) by glycerol-induced rhabdomyolysis, CD24(+)CD133(+) cells regenerated different portions of the nephron, reduced tissue necrosis and fibrosis, and significantly improved renal function without tumor formation [39]. The authors concluded that CD24(+)CD133(+) cells represent a subset of multipotent early embryonic progenitors that persist in human kidneys.

A group headed by Benjamin Dekel employed a unique stepwise strategy in order to identify cell surface markers for isolation of human embryonic renal stem/progenitor cells. To identify candidate markers that are highly expressed in renal progenitor-rich tissues compared with differentiated renal tissues, the group compared the global gene expression in human embryonic kidney, human adult kidney, and Wilms' tumor (WT) by oligonucleotide microarray [40]. Since WT specimens frequently harbor cells that have differentiated into renal tubular epithelia or stroma, in addition to uninduced metanephric blastema, the group serially propagated WT xenografts in mice to purify blastema tissues at the expense of differentiated elements for the analysis. They then pinpointed molecular markers jointly overexpressed in the human embryonic kidney and WT in the microarrays. This analysis uncovered a large number of candidate genes marking renal "stemness," including nephron progenitor transcription factor markers (e.g., *SIX1, SIX2*, *CITED1, EYA1, SALL1*, and *PAX2*) and cell surface markers (*NCAM1*, *PSA-NCAM*, *FZD7*, *FZD2*, *DLK1*, *ITGα8, ACVRIIb*, and *NTRK2*) [40, 41]. The group sought to find the best candidate surface molecule by identifying those that were exclusively expressed in undifferentiated cortical areas of embryonic kidneys; furthermore, they sought to find a surface protein that marked cells that had characteristics of tissue-specific stem cells including those that expressed renal progenitor genes and had self-renewal capacity, colony formation capability, and renal differentiation potential [42]. They eventually identified NCAM1 (CD56) as a potential surface marker for human embryonic renal stem/progenitors that fulfills all these criteria [41, 43].

NCAM1(+) cells isolated from human embryonic kidney cells and cultured in serum-free medium showed significant enrichment of nephron progenitor genes, such as *SIX2*, *OSR1*, *PAX2, SALL1, CITED1*, and *WT1*, compared to NCAM1 (−) cells [43]. The renal differentiation potential of NCAM1(+) cells was demonstrated using a chorioallantoic membrane (CAM) transplantation model, in which cells are grafted onto a highly vascularized niche, rich in developmental cues [44]. The model showed that NCAM1(+) cells generated well-developed epithelial tubular structures expressing markers for the proximal tubule, distal tubule, and loop of Henle. Harari-Steinberg et al. evaluated the regenerative potential of the NCAM1(+) population in a 5/6 nephrectomy CKD model; they demonstrated that NCAM1(+) human embryonic nephron progenitors engrafted and integrated into diseased murine kidneys after transplantation, and that consecutive intraparenchymal administration halted the progression of renal disease and improved creatinine clearance over 12 weeks of treatment [43]. Therefore, it was concluded that NCAM1(+) human nephron progenitors are a pool of mitotically competent yet strongly nephron-generating biased cells. Importantly, the NCAM1(+) cells sorted from human fetal kidney and cultured in serum free media encompassed both SIX2(+) and SIX2(−) cells. Therefore, for enrichment of the SIX2(+) progenitor cell fraction, additional surface markers were required as well as defined media that favors expansion of the SIX2(+) population. In addition, to optimize culture conditions for human cells, the group used media developed initially by the Oxburgh group [36] for expansion of mouse nephron progenitors [45]. It also became apparent that to exclude any renal epithelial differentiation, a combination of positive and negative markers should be employed for prospective isolation [45, 46]. Accordingly, utilizing three markers NCAM1, EpCAM, and CD133 allowed prospective isolation of progenitors [NCAM1(+)EpCAM(−)(CD133(−)] from their differentiating epithelial progeny that arise in development. As such, isolation of early differentiating fetal kidney renal progenitors may also represent a potential regenerative strategy (e.g., by sorting NCAM1(+) EpCAM(dim) cells) [46].

Although the embryonic kidney progenitors represent a promising cell source for renal regeneration, the ethical problems arising from the use of human embryos as well as the allogeneic nature of primary human cells limit their use. In this regard it remains to be determined whether the bio-marker system Dekel and colleagues discovered for prospective isolation of human fetal kidney progenitor cells will be identical in PSC-derived kidney organoids. Therefore, efforts to identify a nephrogenic cell population in adult kidneys or to generate cells equivalent to embryonic renal progenitors from autologous stem cells, such as iPSCs, are required to accelerate research towards the clinical application of transplantation therapy for kidney diseases.

Generation of Kidney Lineage Cells from PSCs

PSCs

PSCs can be broken down into two major types, ESCs [2] and iPSCs [3, 4]. ESCs are derived from the inner cell mass of fertilized eggs in mammals [2], while iPSCs are generated from transducing somatic cells with reprogramming factors, such as the combination of OCT4, SOX2, KLF4, and c-MYC that are found in ESCs [3, 4]. Moreover, regenerative medicine research has explored in vivo transplantation of organ-specific cell types generated from PSCs in vitro. iPSCs are appealing because they offer similar features of ESCs while avoiding the ethical problems related to the use of human fertilized eggs. Further, unlike ESCs, they also can be made from the patients' own cells, which would allow for autologous transplantations, to avoid the risk of immune rejection after the transplantation [47].

Despite the potential of iPSCs for medicine, they have the risk of developing into cancers. iPSCs were originally derived using genome-integrating vectors, such as retroviral and lentiviral vectors, to introduce the reprogramming factors into somatic cells, which could lead to cancer [3, 4]. Although several alternative iPSC derivation methods have been reported to solve the mutagenesis problem, most are less efficient and reproducible than the original methods. Okita et al. developed a safe and efficient system that eliminates the integration of reprogramming factors into the genome of the somatic cells by using episomal vectors encoding OCT4, SOX2, KLF4, L-MYC, LIN28, and a shRNA for TP53 [47]. This method leads to iPSC formation from any cell type with equivalent efficiency to the original methods with less risk of neoplasia.

Clinical trials of cell therapy using human ESC-derived cell types have happened for spinal cord injury, retinal degenerative disorders, type 1 diabetes, and heart failure [48]. The first clinical trial included transplantation of human iPSC-derived retinal pigmented epithelial sheets into a patient suffering from age-related macular degeneration [47]. Thereafter, cell therapies using iPSCs have been used for clinical trials for several other intractable disorders including Parkinson's disease, heart failure, corneal diseases, and thrombocytopenia. In parallel, clinical trials using drug compounds screened for effectiveness in iPSCs derived from patients with hereditary disorders, such as fibrodysplasia ossificans progressiva (FOP), amyotrophic lateral sclerosis (ALS), and Alzheimer disease, are ongoing [47].

Directed Differentiation of PSCs into Kidney Lineage Cells

Although PSCs in kidney research was originally slow compared to use with other organs, substantial progress has recently been made. By recapitulating the signals involved in the differentiation steps during embryonic development, protocols have been used to differentiate PSCs into kidney lineages (Fig. 1a). Kim and Dressler reported that embryoid bodies (EBs) formed from mouse ESCs can be induced into IM in vitro by a combined treatment of RA, activin A, and BMP7 [49]. These EBs developed renal tubule-like structures in coculture with embryonic spinal cord, which is a classical inducer of the MM; furthermore, the induced cells integrated into the proximal tubules when microinjected into E12.5 mouse kidney organ cultures. Taguchi et al. demonstrated that

NPCs are derived from the posterior mesoderm and identified the optimal combination of factors that drive formation of NPCs [15]. They established a stepwise differentiation protocol from mouse ESCs to the epiblast, nascent mesoderm, posterior nascent mesoderm, posterior IM, and ultimately NPCs; these NPCs were capable of forming 3D nephron structures containing numerous glomeruli and renal tubules by coculture with embryonic spinal cords.

Over the last decade, many studies have achieved the directed differentiation of kidney lineages from human PSCs [5, 6]. Mae et al. generated OSR1-GFP knockin reporter human iPSC lines to monitor the expression of OSR1; the group demonstrated that treatment with activin A, BMP7, and CHIR99021 in two-dimensional (2D) monolayer cultures induced the cells into OSR1(+) cells at over 90% efficiency [50]. These induced IM cells could further differentiate into adult renal cell types, such as glomerular podocytes and renal tubular cells, and form 3D renal tubular structures in vitro. Lam et al. also established a simple, rapid, and efficient 2D differentiation protocol from human PSCs into IM with 70–80% efficiency [51]. These IM cells could differentiate into cells expressing NPC markers and gave rise to apically ciliated proximal tubular structures.

Subsequent to the studies with OSR1 reporter lines, the selective induction of NPCs through posterior IM was reported. Taguchi et al. modified the differentiation protocol used to induce mouse ESCs into NPCs (described above) to human iPSC differentiation cultures by changing the early steps for mesoderm induction and the culture periods of the later steps [15]. Although the induction efficiency of NPCs was only 40%, the induced human NPCs formed 3D nephron structures (nephron organoids) that consisted of glomeruli and renal tubules upon coculture with mouse embryonic spinal cords. Takasato et al. reported that prolonged CHIR99021 treatment shifts the fate of human PSCs from anterior IM to posterior IM and that the mixture of these two IM populations spontaneously form self-organized kidney organoids containing multiple kidney cell types, such as glomeruli, renal tubules, collecting ducts, and interstitial and vascular cells [52]. Morizane et al. later reported a simple and efficient 2D culture to differentiate human PSCs into NPCs at around 90% efficiency [53]. More recently, Tsujimoto et al. established a six-step differentiation method that induces multiple human iPSC lines through the primitive steak and posterior IM into NPCs with nephrogenic potential at around 90% efficiency, by more accurately reproducing the developmental process of NPCs (Fig. 1b, c) [13]. By screening the effects of various growth factors in the differentiation culture from the mouse embryonic NPCs to glomerular podocytes, Yoshimura et al. succeeded in generating podocytes from human iPSCs and NPCs at more than 90% efficiency [54]. The induced podocytes exhibited morphologic features, gene expression profiles, and functional responsiveness to drug-induced injury that were comparable to those of adult human primary podocytes.

To induce UB lineages, Xia et al. generated UB progenitor IM cells from human iPSCs that contributed to mouse UB tissues upon coculture with dissociated mouse metanephric cells in organ cultures [55]. Two other groups later reported the directed differentiation of UB-like tissues from PSCs [56, 57]. Taguchi and Nishinakamura induced both mouse ESCs and human iPSCs through anterior IM and nephric ducts (NDs) into 3D UB-like tissues using EBs [56]. However, the differentiation efficiency of the ND epithelia was low (around 40%), requiring purification of the epithelia by flow cytometry sorting before further differentiation into UB-like tissues. Conversely, Mae et al. succeeded in efficiently generating ND epithelia at more than 70% of induction rate by inducing human iPSCs through anterior IM in a 2D monolayer differentiation protocol that avoided the purification step when generating UB-like tissues (Fig. 1d, e) [57]. However, the UB-like tissues generated with either method showed limited capacity for branching morphogenesis. More recently, Mae et al. modified their directed differentiation method to generate UB organoids [58]. They used cultures with low-concentration Matrigel to generate UB organoids that exhibited apicobasal polarity, tubular lumens, and repeated branching potential (Fig. 1f–h). Moreover, the separated tip regions from the organoids were able to continue to repeatedly branch and reconstituted UB organoids. The group repeated the separation process three to four times, allowing each organoid tip to branch four to five times, indicating that each tip had the capacity to branch at least 20 times like their counterparts in human fetal kidneys. Moreover, the group succeeded in further inducing human iPSC-derived UB organoids into collecting duct progenitors expressing AQP2, which corresponded to their counterparts in GW7 embryonic kidneys as evaluated by comparative gene expression (Fig. 1i) [58].

In summary, human PSCs can be differentiated into both NPCs and UB tissues through the IM; furthermore, nephron structures containing glomeruli and renal tubules and collecting duct tissues can be formed in vitro from the induced progenitors.

Kidney Reconstruction

As described above, nephron organoids containing glomeruli and renal tubules and collecting duct organoids can be generated from human PSC-derived NPCs [13, 15, 52, 53] and UB cells [58], respectively. Upon transplantation into the subcapsular or subcutaneous space of immunodeficient mouse kidneys, glomeruli in the nephron organoids vascularized and connected to the host's blood stream [32, 59–61]. The transplanted glomeruli formed slit diaphragm-like structures consisting of the basement membrane, fenestrated endothelial cells, and podocyte foot processes [59]. The transplanted tubular epithelia also exhibited polarization and segmental specialization, suggesting that functional vascularization facilitates glomerular and tubular maturation in vivo. Furthermore, in vivo imaging showed that glomeruli in the transplanted human PSC-derived nephron organoids displayed dextran and albumin size selectivity across their glomerular filtration barrier, and proximal tubules reabsorbed dextran, indicating functional filtration of the transplanted human glomeruli [60].

Taguchi and Nishinakamura combined NPCs and UB tissues differentiated from mouse ESCs and stromal progenitors removed from mouse embryonic kidneys in vitro, which then formed renal organoids with glomeruli, renal tubules, and collecting ducts that were interconnected [56]. Tsujimoto et al. generated human kidney organoids by combining human iPSC-derived NPCs and UB cells in vitro, leading to interconnections between NPC-derived glomeruli and renal tubules and UB-derived collecting ducts (Fig. 1j) [13]. Upon transplantation of these organoids into the renal subcapsular space of immunodeficient mice, connections between human iPSC-derived glomeruli and the host's blood stream were observed by intravital multiphoton microscopy imaging (Fig. 1k, l). Limitations however are that these kidney-like structures generated from human PSCs were very small in size at only several millimeters in diameter. Thus, generation of larger kidney tissues are required for clinically relevant use in human kidney transplantation.

Towards this goal, an experimental strategy called interspecific blastocyst complementation has been investigated, in which wild-type PSCs are injected into the blastocysts of animals genetically modified not to develop the target organs of interest and thus the organs are formed entirely from the injected PSCs. Nakauchi's group demonstrated that wild-type mouse and rat PSCs injected into PDX1 (−/−) (pancreatogenesis-disabled) rat and mouse blastocysts, respectively, generated an almost entire PSC-derived pancreas interspecifically [62]. This approach has subsequently been applied to generate whole kidneys. Usui et al. injected wild-type mouse ESCs into the blastocysts of *Sall1* knockout mice and confirmed that kidneys developed with MM-derived glomeruli and renal tubules that originated from the injected ESCs [63]. However, as expected, the UB-derived collecting ducts and lower urinary tract and vasculature were derived from the host mouse. Goto et al. recently succeeded in generating mouse PSC-derived MM in anephric SALL1 (−/−) mutant rat by interspecific blastocyst complementation [64].

Yamanaka et al. utilized the developmental organ niche of later stage fetuses of different species for kidney organ regeneration [65]. They developed a combination system by which donor NPCs are precisely injected into the nephrogenic zone of Six2-Cre-inducible diphtheria toxin (DT) receptor transgenic mice, in which native

NPCs are conditionally eliminated by DT injection. They succeeded in removing Six2(+) NPCs in the nephrogenic niche of the host mice and replacing them with transplanted wild-type mouse NPCs in ex vivo organ cultures. The transplanted NPCs developed into glomeruli and renal tubules that connected to host collecting ducts and further vascularized following transplantation. Furthermore, they interspecifically generated rat nephrons by replacing host mouse NPCs [65]. Later, the group developed a cell transplantation method through which mouse NPCs were accurately transplanted into the fetal kidney development area of E13–14 mouse embryos in utero [66]. The transplanted NPCs contributed to the chimeric CM of donor and host NPCs that was connected to the host UB. This experimental approach also generated chimeric kidneys in which MM lineage cells originate from donor PSCs while other kidney constituents are from the host animals. Further studies may develop strategies to replace both MM and UB lineage cells with donor PSC-derived cells to generate kidneys that are entirely from PSCs.

Cell Therapies Using Human PSC-Derived Kidney Progenitors

In parallel with reconstruction of the kidney organs, human iPSC-derived kidney progenitors have been used as cell therapies to treat kidney diseases [67, 68]. Imberti et al. generated and injected NPC-like progenitors from human iPSCs into the tail vein of immunodeficient mice with AKI induced by cisplatin administration [67]. This therapy significantly suppressed the elevation of blood urea nitrogen (BUN) and the histological injury typical of AKI, such as tubular necrosis and cast formation in the host mice. Because the injected progenitors only integrated into and contributed to small portions of the recipient's renal tubules, the therapeutic effects might be attributable to the paracrine effects by humoral factors secreted from the progenitors.

Toyohara et al. generated OSR1-GFP/SIX2-tdTomato double knockin reporter human iPSC lines and induced the cells into OSR1(+)SIX2(+) NPC-like progenitors [68]. They transplanted the OSR1(+)SIX2(+) progenitors after sorting by flow cytometry into the renal subcapsular space of immunodeficient mice with AKI induced by I/R injury with unilateral nephrectomy. Increased BUN and serum creatinine values of the host mice were significantly suppressed compared with saline injection or transplantation of undifferentiated human iPSCs (Fig. 2a). The therapy also significantly ameliorated the histological injury patterns of AKI, including tubular necrosis, urinary casts, and interstitial fibrosis (Fig. 2b). The transplanted progenitors had not integrated into host kidney parenchyma, however, suggesting again that the therapeutic benefits were mainly produced by paracrine factors secreted from the progenitors. Indeed, the study also showed that the progenitors secreted multiple known renal trophic factors, such as angiopoietin 1, vascular endothelial growth factor (VEGF) A, and hepatocyte growth factor (HGF) [68].

The therapeutic benefits of mouse embryonic NPCs with AKI were also confirmed recently. Li et al. showed that the renal subcapusular transplantation of mouse embryonic NPCs expanded in vitro improved survival rate and ameliorated kidney injury in cisplatin-induced AKI model mice both biochemically and histologically. The transplanted NPCs did not integrate into the parenchyma of the host mouse kidney, suggesting once more that paracrine factors likely produce the beneficial effects [38].

Embryonic Renal Stem/Progenitor Cells Reprogrammed from Adult Renal Cells

The success of direct cellular reprogramming from one terminally differentiated cell type into another has been shown in several cell types including kidney lineage cells [5, 6]. In these reports, transduction using a combination of three or more transcription factors was essential for differentiation into the target cell type. Kaminski et al. converted the fibroblasts to an induced renal epithelial cell (iREC) via the combined expression of *Emx2*, *Hnf1b*, *Hnf4a*, and *Pax8* [69]. Hendry et al. reported that the forced expression of six transcription factors

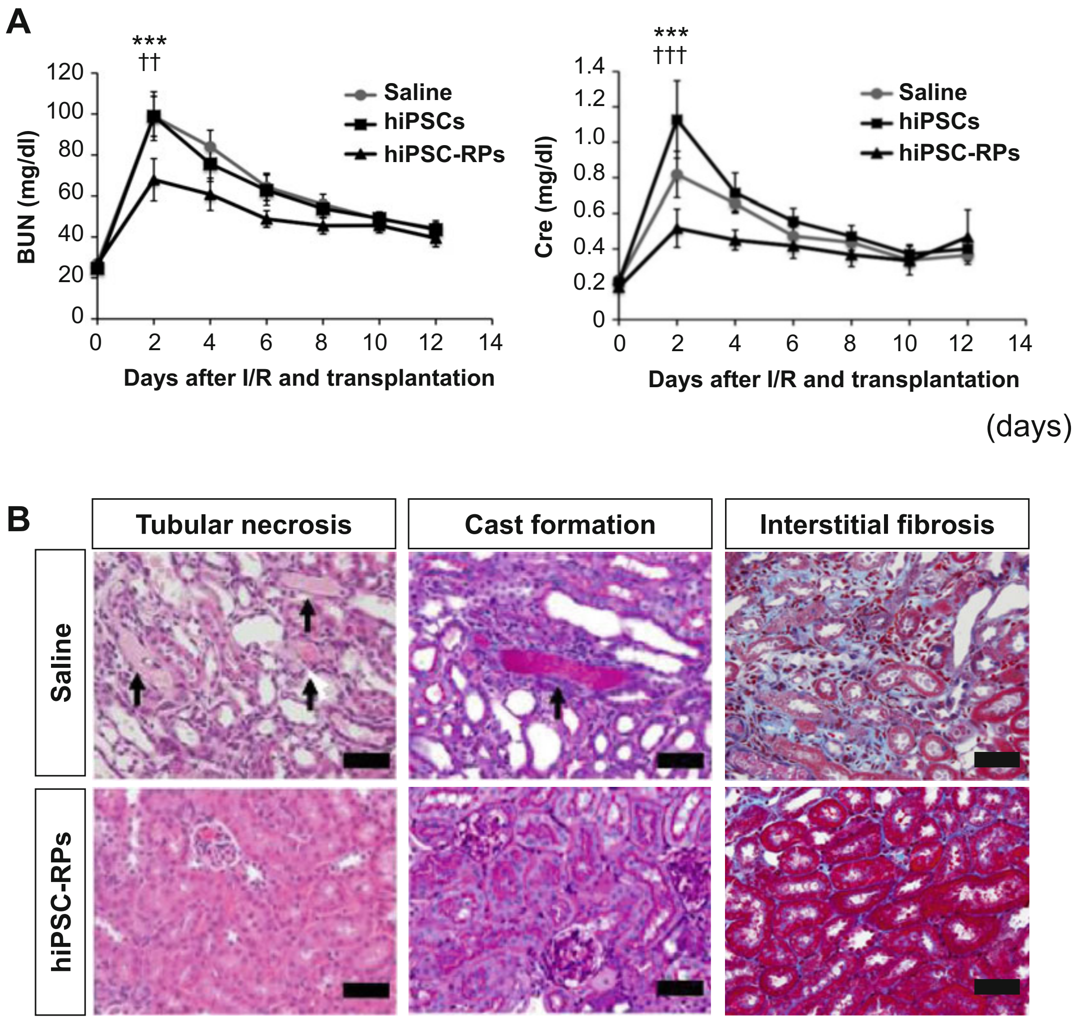

Fig. 2 Cell therapy using human iPSC-derived kidney progenitors. (**a**) Time course analysis of blood urea nitrogen (BUN) and serum creatinine (Cre) values in acute kidney injury (AKI) model mice induced by ischemia-reperfusion (I/R) injury that received saline injection (grey circles) or the renal subcapsular transplantation of undifferentiated human iPSCs (hiPSCs; black squares) or of human iPSC-derived renal progenitors (hiPSC-RPs; black triangles). ***, $p < 0.001$ versus saline; ††, $p < 0.01$ versus hiPSCs; †††, $p < 0.001$ versus hiPSCs. (**b**) Representative sectional images of host mouse kidneys that received the saline injection (upper panels) or the transplantation of hiPSC-RPs (lower panels) and stained with hematoxylin and eosin (HE; left panels), periodic acid-Schiff (PAS; middle panels) or Masson's trichrome (MT; right panels) on day 12 after the I/R injury and transplantation. Arrows indicate tubular necrosis in the upper left panel and cast formation in the upper middle panel. Scale bars, 20 μm. (Adapted from Toyohara et al. [68])

(*SIX1*, *SIX2*, *OSR1*, *EYA1*, *HOXA11*, and *SNAI2*) or even just three (*SIX1*, *OSR1*, and *SNAIL2*) drove reprogramming to an NPC-like fate [70, 71]. Hiratsuka et al. directly introduced synthetic mRNAs encoding transcription factors into human PSCs to generate NPC-like cells (*FIGLA*, *PITX2*, *ASCL1*, and *TFAP2C*) or to become pretubular aggregates (*HNF1A*, *GATA3*, *GATA1*, and *EMX2*; [72]).

In addition, Omer et al. showed that treating mature human adult kidney epithelial cells with valproic acid, a histone deacetylase (HDAC) inhibitor, results in significant activation of embryonic renal progenitor genes, such as SIX2, OSR1, SALL1, NCAM, and PSA-NCAM [73]. However, the same treatment inhibits cell dedifferentiation towards a more replicative mesenchymal state, which is required for the clonogenic expansion and acquisition of stem cell characteristics. The authors are now utilizing single pluripotent molecules such as OCT4 to achieve a tissue progenitor state in cultures of human adult kidney to allow both clonogenic self-renewal and preservation of renal potential over many generations.

In summary, research on direct cellular reprogramming into renal cell types is still nascent, and further studies will be required to establish methods for generating renal stem/progenitor cells that are devoid for any risk of malignant transformation.

Adult Renal Stem/Progenitor Cells

Stem Cells of Extrarenal Sources

Two types of tissue-specific stem cells included in bone marrow have been reported to contribute to renal tissues: hematopoietic stem cells [74] and mesenchymal stem/stromal cells (MSCs: [75]). While originally hypothesized to be circulating from bone marrow and responsible for the homeostasis, regeneration and repair of adult mesenchymal tissues, MSCs are now considered a subpopulation of perivascular cells or pericytes that reside in almost all tissues of our body. MSCs are thought to be recruited from their perivascular niche to sites of tissue injury, where they establish a regenerative microenvironment to facilitate tissue regeneration by secreting various bioactive molecules [76].

Early reports described that bone marrow–derived cells can differentiate into multiple somatic cell types including renal tubular cells, indicating the "plasticity" of stem cells to transdifferentiate into cells belonging to other organs, including organs derived from other germ layers [77]. However, since those reports, spontaneous cell fusion was demonstrated between mouse bone marrow cells and mouse ESCs [78], suggesting that at least part of the transdifferentiation of bone marrow stem cells occurs by spontaneous cell fusion between transplanted bone marrow cells and recipient host tissue cells.

The injection of bone marrow–derived cells, especially MSCs, has repeatedly been shown to improve renal function in AKI induced by toxins (cisplatin and glycerol) or I/R injury [79]. Similar beneficial effects on renal function may be obtained by administering growth factors, such as granulocyte colony-forming factor (G-CSF), granulocyte/monocyte colony-forming factor (GM-CSF), monocyte colony-forming factor (M-CSF), or stem cell factor, to mobilize a patient's bone marrow cells. Several studies (except for one) have indicated such administration improves renal function.

It is currently believed that any contribution made by nonrenal stem cells, including MSCs, to the regeneration of renal tissues is not due to differentiation and integration into tubular cells in vivo. Humphreys et al. elegantly demonstrated this concept in mice by using lineage tracing experiments [22]. They demonstrated that the cells responsible for tubular regeneration after AKI induced by I/R injury originate from remaining renal tubular cells, thereby excluding any possible contribution of extrarenal stem cells to regenerating kidney. Instead, MSCs seem to contribute to kidney repair after damage by secreting trophic factors, such as VEGF, bFGF, monocyte chemoattractant protein-1 (MCP-1), HGF and insulin-like growth factor 1 (IGF1), and immunomodulators, such as TGF-β and prostaglandin E2 (PGE2). While MSCs have consistently been shown to exert renoprotective effects in animal models of AKI, the therapeutic effectiveness of MSCs in preventing CKD is controversial [79].

A novel strategy mixing extrarenal and renal tubule-forming cells has recently been suggested as a potential route to harness the relative advantages of each cell type. Pleniceanu et al. have generated a cellular mix for regeneration; they combined human vessel-forming cells, comprised of endothelial colony-forming cells (ECFCs) and MSCs, with kidney tubule-forming cells resulting in 3D human kidney spheroids termed nephrospheres. Transplantation of nephrospheres into the subcapsular space or directly into the kidneys of mice resulted in the formation of both renal tubules and patent donor-derived vessels, which connected to the host vasculature and carried host RBCs [80]. This strategy has the benefit of generating renal parenchymatic units (i.e., tubules), as well as new vessels, which together have potential in improving oxygenation to the hypoxic CKD kidney and alleviating tissue fibrosis.

Sources of Adult Renal Cells Responsible for In Vivo Regeneration

As mentioned above, the lineage tracing experiments done by Humphreys et al. demonstrated that the cells responsible for tubular regeneration after I/R injury are of tubular origin, not transplanted extrarenal cells [22]. The study also demonstrated that adult mouse kidneys do not contain embryonic NPC-like cells expressing *Six2*, and that the reactivation of this cell population does not occur even after ischemic damage. This indicated that regeneration of new tubular cells originates from intrinsic epithelial cells in adult kidneys. However, it remains unclear whether these regenerating cells are epithelial progenitors with more restricted differentiation potential than Six2(+) embryonic progenitors or are fully differentiated tubular cells that can expand after ischemic damage.

Kusaba et al. answered this question by genetically labeling fully differentiated proximal tubule cells using mice in which a CreER(T2) cassette was knocked into the sodium-dependent inorganic phosphate transporter SLC34a1 locus; they then examined the cells' contributions to regenerating renal epithelia after ischemic injury [81]. After injury, all tubular cells remained labeled, indicating that new regenerating tubular cells originated from the proliferation of surviving differentiated tubular epithelia. Interestingly, injury to labeled proximal tubule epithelia induced a certain degree of dedifferentiation as manifested by the marker expression of putative epithelial stem cells, such as CD24, CD133, vimentin, and kidney injury molecule-1 (KIM-1). Their results provided no evidence for an intratubular stem cell population, but rather indicated that terminally differentiated tubular epithelia dedifferentiate to some extent by reexpressing some stem cell markers to then repair the damaged tubules [81].

Consistent with these results, Vogetseder et al. investigated the contribution of stem cells to the growth of proximal tubular S3 segments in healthy rats by administering thymidine analog and bromodeoxyuridine (BrdU) and examining expression of cell cycle markers, such as the G(1)-phase marker cyclin D1 [82, 83]. They found that cycling and non-cycling tubular cells have the same degree of differentiation, that the transient amplifying cells, usually thought to be the descendent of stem cells, were absent, and that most tubular cells in the S3 segment divided or entered the cell cycle in response to a proliferative stimulus. These results confirmed that tubular cells originate from differentiated tubular cells and not from stem cells [82]. The group also demonstrated that ~40% of S3 tubular cells are in G(1) phase and that a strong mitotic stimulus shortens the period of quiescence following cell division; together, this suggested that the capacity of the proximal tubule to rapidly recruit cells to divide depends on a large reserve pool of cells in G(1) cells with capacity to shorten the quiescence period [83].

Similar results were demonstrated by Berger et al. using the transgenic PEC (parietal epithelial cell)-reverse-tetracycline trans-activator (rtTA) mouse, which allowed cell fate tracing of regenerating proximal tubular cells [84]. They found that tubular regeneration after I/R injury occurred from surviving tubular cells rather than from fixed progenitor cells. Together, these aforementioned studies strongly suggest that no resident pool of stem cells exist to regenerate lost tubular cells after AKI, but rather surviving tubular cells proliferate to repair the injury.

Rinkevich and Dekel examined the mechanisms and magnitude by which the mammalian kidney generates and maintains tubular cells by using multicolored "rainbow" mice, in which every cell is randomly marked by one of four fluorescent colors, allowing long-term lineage tracing and clonal analysis of new tubular cells [85]. They found that the adult mammalian kidney undergoes constant cell replacement during homeostasis and after injury by clonal expansion of lineage – or fate – restricted precursor cells that give rise only to cells of the same nephron segments they reside in.

Wnt activation in single cells could initiate the largest clones. They concluded that adult nephron epithelial cells can function as uni-potentional progenitors continuously maintaining and preserving all segments of the mouse kidney throughout life by clonal proliferation (Fig. 3). Interestingly, the same lineage boundaries within the kidney were found to apply to in vitro cultured cells. For instance, Cohen-Zontag et al. recently identified single-cell-derived clones via transcriptomics, and demonstrated ex vivo human kidney cell growth in segment-specific clones harboring either proximal or distal identity, as opposed to mixed clones [86]. This confirms both the lack of multipotent stem cells in the adult mammalian kidney and existence of clear sublineage boundaries in its in vitro equivalent. Notably, similar findings have been demonstrated by Pleniceanu et al. with respect to nonepithelial lineages in the kidney [80]. In order to generate both epithelial tubules and human vessels upon injection into the murine renal subcapsules, three cell types were required: renal tubule-forming cells, such as kidney spheroids, endothelial progenitors (ECFCs), and MSCs which are needed to generate pericytes. Taken together, these studies indicate that for successful regeneration of renal parenchyma and renal vessels in a diseased kidney, transplantation of mixed cell types will be required.

In summary, the origin of regenerating tubular cells is from fully differentiated tubular epithelia, but at the same time these cells may acquire some features of unipotent epithelial stem-like cells that can clonally expand and become replication competent under specific proliferation stimuli. However, most studies examining the origin of tubular regeneration have been performed in AKI mice models induced by I/R injury. It is possible that different regenerative mechanisms may be involved in other kidney injury models.

Adult Renal Stem/Progenitor Cells Expanded Ex Vivo

Many studies have characterized adult renal stem/progenitor cells using in vitro cultures. These cell populations might result from cell-culture-

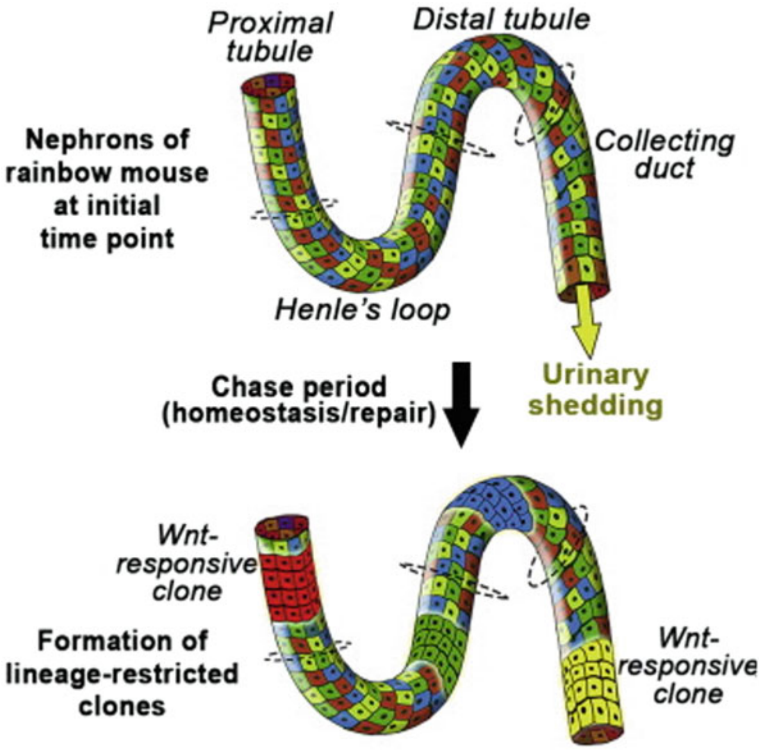
Proximal tubule
Distal tubule
Nephrons of rainbow mouse at initial time point
Collecting duct
Henle's loop
Chase period (homeostasis/repair)
Urinary shedding
Wnt-responsive clone
Formation of lineage-restricted clones
Wnt-responsive clone

Fig. 3 Cartoon showing how renal tubular cells regenerate during steady state and after injury at the clonal level. “Rainbow” mice permanently label individual kidney cells from embryonic development and throughout adult life (top). Lineage restricted clones that are fated to proximal, distal, or collecting duct tubules renew and regenerate the mouse kidney throughout life (bottom). (Adapted from Rinkevich et al. [85])

induced changes in morphology, behavior, and marker expression, although they are still possibly clinically useful. Development of methods to expand nephron-forming cells from adult kidney while preserving stem/progenitor function could supply a source for cell therapy.

Renal stem/progenitor cells have been reported to exist in or were isolated from various locations in adult kidneys, including the renal papilla, interstitium, tubular epithelial cells, Bowman's capsule, decapsulated glomeruli, juxtaglomerular apparatus, and collecting ducts. They have been identified with various assays, such as label-retaining assays, clonogenic assays, sphere or spheroid assays, flow cytometry using cell surface markers, lineage tracing studies, and side population (SP) assays [87].

Label-retaining cells (LRCs) identified in vivo by BrdU labeling exhibit a characteristic slow cycling of tissue-specific stem cells. Maeshima et al. showed that LRCs are found in renal epithelial tubular cells of rat kidney and that they reentered mitosis and differentiated into tubular epithelia in response to renal ischemia; thus, contributing to tubular regeneration after damage [88]. Oliver et al. identified a population of LRCs in the interstitium or upper papilla of adult rodent kidneys [89]. These LRCs can rapidly proliferate in response to ischemic insult, suggesting that they may play a role after acute injury. However, the LRCs from these reports might represent transiently amplifying cells rather than stem cells. Proof of the existence of a pluripotent adult renal stem cell population with long-term self-renewal capacity and clonogenicity remains to be shown.

Kitamura et al. screened for stem cells in various regions of the postnatal rodent kidney by the dissection of various nephron segments and culture after dissociation to single cells [90]. They established clonally expanded cells from the S3 segment of rat proximal renal tubules, which are known to be most susceptible to injury and to have the highest proliferation rate in nephron segments. The established cell line showed features of stem cells and could be maintained long term without transformation.

Buzhor et al. was the first to establish 3D spheroid cultures of human kidney epithelial cells retrieved from human nephrectomy tissue samples by suspension cultures in low-attachment conditions [91]. The human kidney spheroids upregulated certain renal developmental and "stemness" markers, including *PAX2*, *SALL1*, *SIX2*, *WT1*, *NANOG*, and *GPC3*, and indicated mostly an EpCAM(+)CD24(+)CD133(+)CD44(+) phenotype. When grafted in low cell numbers onto the CAM of chick embryos, the spheroid cells exclusively reconstituted renal tubular epithelia of different nephron segments. More recently, Harari-Steinberg et al. grew 2D cultures from primary human kidney cells and then cultured them as 3D spheroids, finding that the renal epithelial identity lost in 2D culture conditions was reestablished; furthermore, upon grafting of human kidney spheroids into CKD mice, long-term engraftment and differentiation into kidney structures in mice was achieved and kidney function improved [92]. In addition, the kidney spheroids were shown to confer an antifibrotic paracrine effect, as evident in transwell experiments, by downregulating collagen production from cocultured fibroblasts. Notably, the same affect was obtained when using kidney spheroids derived from kidneys from patients with ESRD (Fig. 4).

Bombelli et al. reported a modified sphere-formation protocol to generate nephrospheres from normal human adult kidneys [93]. The cultured nephrospheres activated stem cell pathways, contained cells at different levels of maturation, and generated 3D tubular structures both in vitro and in vivo. The group identified a quiescent PKH^{high} stem cell population within the spheres that demonstrated in vitro self-renewal capacity and differentiation potential into epithelial, endothelial, and podocyte progenies.

Bussolati et al. isolated tubule cells expressing a hematopoietic stem cell marker, CD133, from human renal cortex [94]. These cells expanded, differentiated in vitro into epithelial or endothelial cells and showed developmental potential to form tubule-like structures and functional vessels. They were also incorporated into tubules when intravenously injected into immunodeficient mice with AKI induced by glycerol-induced rhabdomyolysis.

Dekel et al. identified nontubular cells that expressed the stem marker SCA1 as MSC-like progenitor cells in the papillary interstitial space of the

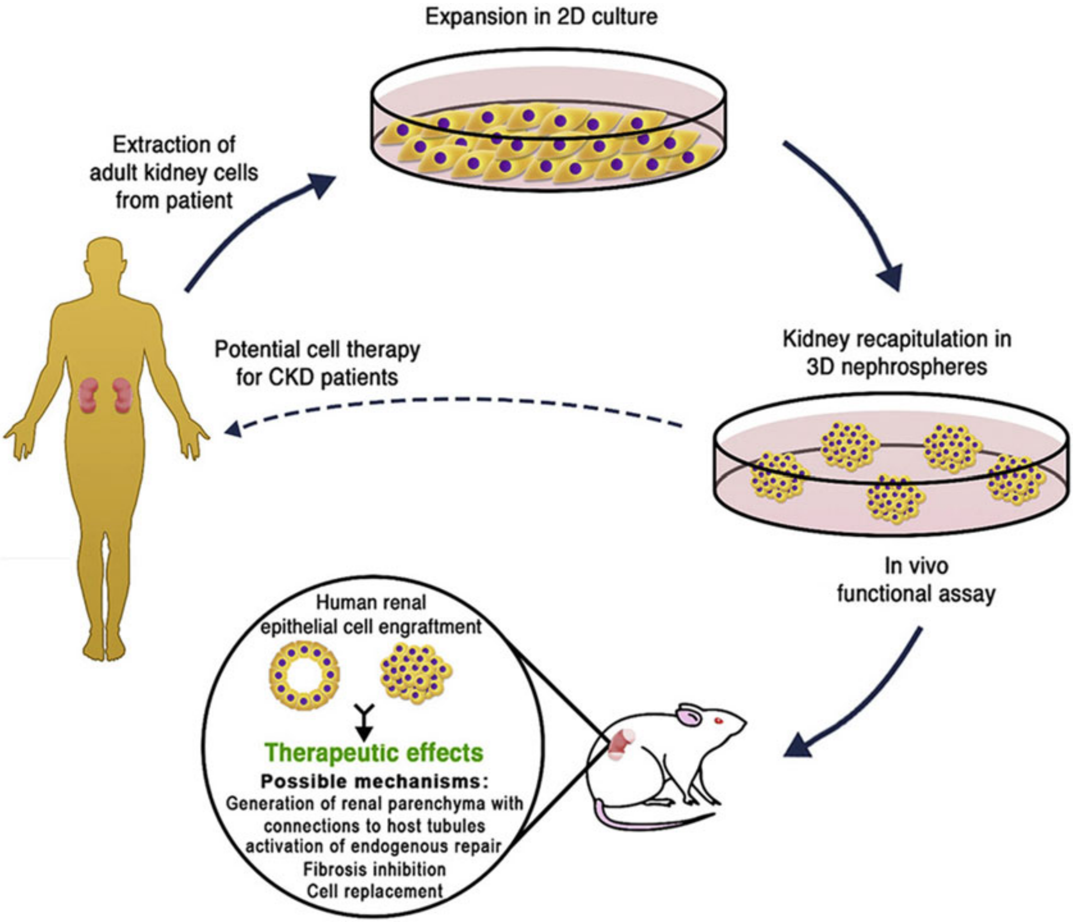
Expansion in 2D culture
Extraction of adult kidney cells from patient
Kidney recapitulation in 3D nephrospheres
Potential cell therapy for CKD patients
In vivo functional assay
Human renal epithelial cell engraftment
Therapeutic effects
Possible mechanisms:
Generation of renal parenchyma with connections to host tubules
activation of endogenous repair
Fibrosis inhibition
Cell replacement

Fig. 4 Scheme of potential future use of kidney spheroids as an autologous cell treatment. Kidney cells extracted from a patient with CKD are first expanded in monolayer (2D) conditions, and then transferred into 3D-promoting culture conditions to obtain nephrospheres. These, in turn, can serve as the starting point for both autologous cell treatment, and for various in vivo assays. (Adapted from Harari-Steinberg et al. [92])

adult mouse kidney [95]. The cloned renal SCA1(+) LIN(−) cells showed in vitro differentiation potential for myogenic, osteogenic, adipogenic, and neural lineages; the SCA1(+)LIN(−) cells also adopted a tubular phenotype when injected directly into the renal parenchyma of mice with AKI induced by I/R.

A group led by Paola Romagnani has reported that a population of CD24(+)CD133(+) cells localized at the urinary pole of the Bowman's capsule in adult human, mouse, or rat kidneys represent renal progenitors that give rise to both glomerular podocytes and renal tubular epithelia. Clonally expanded CD24(+)CD133(+) cells also contributed to the regeneration of renal tubular structures and significantly ameliorated kidney damage after their intravenous injection into immunodeficient mice with AKI induced by rhabdomyolysis [96].

Bruno et al. found that human glomeruli deprived of Bowman's capsule contain a population of CD133(−)CD146(+) cells that express MSC markers and renal stem cell markers, CD24 and PAX2 [97]. These MSCs exhibit self-renewal capability, clonogenicity, and multipotency capable of osteogenic, adipogenic, and chondrogenic differentiation. In addition, they are able to differentiate into endothelia, epithelial cells expressing podocyte markers and mesangial marker-expressing cells.

Buzhor et al. established a 2D and low-density clonal culture of epithelial cells from adult kidney tissue of nephrectomized patients, in which the reactivation an embryonic mesenchymal marker, NCAM1, was observed in a specific cell population that attained a stem/progenitor state [98]. The isolated NCAM1(+) cells showed proximal tubular origin, expressed early nephron progenitor markers, such as PAX2, SALL1, SIX2, and WT1, and acquired a mesenchymal fate, indicated by high VIMENTIN and reduced E-CADHERIN expression levels. The cell population showed clonogenic potential as single cell cultures, generated 3D kidney spheroids, and differentiated into tubular structures when grafted onto the CAM of chick or into the subcutaneous space of immunodeficient mice. Importantly, NCAM1 is a molecular marker identified in vitro in linage-restricted adult kidney clones assigned to proximal tubule origin [86].

Gupta et al. isolated multipotent renal progenitor cells (MRPCs) from adult rat kidneys by using low-density culture conditions for 4–6 weeks that were similar to those used for the culture of bone marrow–derived multipotent adult progenitor cells [99]. These MRPCs expressed OCT4 and PAX2 and differentiated into tubular structures in vitro and in vivo. However, these cells displayed spindle-shaped morphology, suggesting nontubular and possibly extrarenal origin.

Pippin et al. performed lineage tracing experiments to show that cells of renin lineage in the juxtaglomerular compartment serve as progenitors of glomerular podocytes and parietal epithelial cells in experimental glomerular disease [100]. After a decrease in podocyte number by cytotoxic anti-podocyte antibody, a significant increase in the number of labeled cells of renin lineage was observed in glomeruli; the labeled cells were distributed along Bowman's capsule, within the glomerular tuft, or in both locations, and expressed markers for glomerular parietal cells or podocytes.

SP cells are defined as a cell population that can rapidly efflux DNA-binding dyes, such as Hoechst 33342 and Rhodamine 123, and may exhibit stem cell–like characteristics, including the expression of specific membrane transporters associated with stemness. SP cells in the bone marrow overlap with hematopoietic stem cells, and have also been isolated from other tissues, including the adult kidney [101]. Challen et al., used stringent isolation procedures and found that renal SP cells represent 0.1% of the kidney. These cells showed multilineage differentiation potential into osteocytes and adipocytes in vitro, and into several nephron segments in vivo at low efficiency after their injection into an adriamycin-induced acute glomerular injury model. However, despite stringent selection, the cell population remained heterogeneous, including a monocytic fraction, which may result in the apparent phenotypic plasticity. The injection of adult kidney SP cells did not show significant tubular integration in acute renal damage models. The SP population derived from other organs than bone marrow, including kidneys, does not always show characteristics of tissue-specific stem cells.

Lee et al. isolated mouse kidney progenitor cells (MKPCs) with MSC properties that reside in the mouse kidney interstitium of the medulla and papilla [102]. MKPCs displayed features including spindle-shaped morphology, self-renewal of more than 100 passages without evidence of senescence, and plasticity; they also demonstrated multipotent differentiation potential into endothelial cells and osteoblasts in vitro and endothelial and tubular epithelial cells in vivo. Intrarenal injection of MKPCs in immunodeficient mice with I/R injury rescued renal damage both histologically and functionally and reduced mortality; some of the MKPCs formed vessels with RBCs inside and incorporated into renal tubules.

Li et al. identified an MSC-like population in the adult mouse collecting duct that undergoes an epithelial-to-mesenchymal transition to form clonogenic, long-term self-renewal and retains a capacity to form epithelial structures in vitro and in vivo [103]. After extensive passages, these kidney MSC-like cells selectively integrated into the AQP2(+) medullary collecting duct when microinjected into the kidneys of neonatal mice. Further studies revealed that a *Wnt4*-expressing interstitial population intercalates into the collecting duct in neonatal kidney, giving rise to the MSC-like cells.

Finally, the Clevers group utilized the adult human kidney to expand 3D epithelial structures that recapitulate aspects of the kidney of origin. They found that WNT pathway stimulation leads to long-term growth of primary tubular epithelial organoids termed tubuloids. Human tubuloids which originate from an adult kidney cell mix which operatively can be viewed as stem/progenitor cells represent proximal as well as distal nephron segments and can be used to model infectious and hereditary kidney disease in a personalized medicine platform [104].

In summary, various types of adult renal stem/progenitor cells have been identified by different methods. Although these adult stem/progenitor cells have been reported to contribute to the epithelia of glomeruli and renal tubules like embryonic progenitors, they may represent a different cell population from embryonic NPCs and possess different gene expression profiles and cellular origins. Detailed characterization of these cells and examination of the therapeutic potential of each stem/progenitor cell population are still pending.

Stem Cell Niche in Adult Kidney

Stem cells are usually located in specific anatomic locations termed “niches,” which regulate how stem cells participate in tissue generation, maintenance, and repair [105]. Stem cells are typically located in deep, narrow tissue niches, while transiently amplifying precursor cells and differentiated cells at various stages of maturation lie in the progressively more distal cell layers. In addition to heterologous cellular components, any given niche is thought to consist of an extracellular matrix and other noncellular constituents. Both paracrine and metabolic factors could also serve as a niche, since they respond to exogenous signals via a sensing system consisting of the nervous and/or circulatory system. Accordingly, the perivascular site is considered a stem cell niche [76].

Other studies have indicated that the MSC compartment, which resides in the connective tissue of most organs (including kidneys), is capable of differentiating into osteoblasts, adipocytes, and chondrocytes, as a result of its perivascular location. Based on similar marker genes, such as CD146, and differentiation potential into osteoblasts, adipocytes, and chondrocytes, it has been hypothesized that vascular pericytes may represent the MSC population within any given organ. Indeed, pericytes are quiescent, slow-cycling cells at baseline; however, in the angiogenic stage they show high proliferative potential, capacity for self-renewal, and the ability to generate osteoblasts, chondroblasts, fibroblasts, and pre-adipocytes in vitro, consistent with adult stem cell characters [76].

The microvasculature of the renal papilla is particularly rich in pericytes that regulate microvascular integrity in the peritubular capillary network and give the descending vasa recta, the arteriolar segments supplying blood to the medulla, their contractile function. Therefore, the MSC compartment residing in the renal papilla might be linked to the perivascular niche. Consistent with this hypothesis, Lee et al. [102] identified MKPCs with MSC properties that reside in the mouse kidney interstitium of the medulla and papilla in close association with endothelial cells. Similarly, a report by Oliver et al. [89] showed LRCs with several characteristics of adult stem cells in the papillary interstitium and collecting duct. Finally, Dekel et al. [95] demonstrated that nontubular SCA1(+) multipotent progenitor cells are found mainly in the papillary interstitial space of adult mouse kidney.

In summary, the renal papilla has been a candidate stem cell niche in kidney. However, there are relatively little data on kidney stem cell niches compared with other organs. Thus there is still much to learn about the identify of these niches and their mechanisms for regulating renal stem cells.

CSCs in Kidney

CSCs drive tumor growth by their self-renewal, as well as by giving rise to a large population of differentiated progeny that make up the majority of cells in a tumor [7–9]. Their ability to initiate tumor growth when grafted into immunocompromised mice demonstrates that CSCs exist in a variety of human tumors. Specific signaling pathways play a functional role in self-renewal and/or differentiation of CSCs, suggesting targeted therapies are possible. In addition, identification of markers that enable isolation of CSCs, which are a minority population of tumor-constituting cells, from whole tumor tissues should help clarify the biological properties of CSCs. To date, relatively a small number of studies have reported the identification of renal CSCs [106].

CSCs in Adult Renal Cell Carcinoma (RCC)

RCC is a common form of urologic tumors, accounting for approximately 2% of total human malignancies and >90% of cancers in the kidney with an annual increasing incidence [107]. Major subtypes include clear cell RCC, which is the most common form, papillary RCC, and chromophobe RCC; the remaining subtypes are very rare. Although marked advances in treatment of RCC have been made over the past decade due to earlier detection and increased treatment options, including targeted agents against VEGF and mTOR pathways and immunotherapies, advances in diagnosis, local management, and systemic therapy based on the molecular biology of RCC are needed to achieve better long-term survival. RCC is considered to originate from a mutated tubular population with stem/progenitor cell properties, much like in other organs [106]. Renal CSCs have been isolated from RCC using different stem cell markers or by functional approaches similar to other stem cell isolation methods, including sphere formation or SP assays.

Bussolati et al. identified a population of tumor-initiating cells from RCC and renal undifferentiated carcinomas by means of cell sorting with a mesenchymal marker, CD105 [108]. These cells were cloned in stringent culture conditions and displayed tumor-initiating ability, generating serially transplantable carcinomas. Functionally, CD105(+) cells are clonogenic and able to generate spheres. Moreover, these cells differentiate into epithelial cells in vitro and in vivo to recapitulate the histological pattern of the tumor. Interestingly, CD105(+) cells do not express CD133, which is a candidate marker for adult renal progenitors and also expressed in CSCs of tumors in other organs, such as the nervous system, colon, prostate, pancreas, and lungs [106]. While CD105 has been proposed as the main marker for CSCs in RCC, many studies report that CD105(−) cells also show CSC-like features [106].

Zhong et al. selected cells from a renal cancer cell line, SK-RC-42, that have the ability to form spheres in serum-free medium supplemented with EGF and bFGF [109]. The cell population expressed several “stemness genes,” including OCT4, BMI, β-catenin, and Nanog, showed resistance to chemotherapeutic agents and irradiation, and have higher in vivo tumorigenicity than monolayer adherent cells. Addla et al. used the SP assay to identify renal CSCs [110]. SP cells isolated from renal carcinomas expressed mesenchymal markers including CD105, but not CD133, and showed high proliferative potential and an ability to give rise to differentiated spheroids. Other candidate stem cell markers, such as CD133, CXCR4, and CD44, and enzymatic activity of aldehyde dehydrogenase 1 (ALDH1) have also been used to isolate and characterize CSCs in RCC [106]. However, the detailed characteristics and roles of these cell populations in carcinogenesis or their clinical significance are unknown.

CSCs in WT

WT, also called nephroblastoma, is the most common type of kidney cancer in children and fourth most common among all childhood cancers [111]. WT is typically composed of three elements: undifferentiated blastema, stroma, and tubular structures at varying levels of differentiation, which together resemble the nephrogenic zone of a normal developing kidney. Thus, it is widely accepted that WT arises from transformed renal stem cells, which maintain the undifferentiated blastemal component of the tumor and which maldifferentiate to give rise to tubular elements. Thus, while embryonic nephrogenesis is driven by normal renal stem cells, WT is propagated by an equivalent transformed population, termed WT

CSCs [112]. Reports suggested that nephrogenic differentiation in the tumor was deranged, as the transcription factors that normally specify renal stem/progenitor cells (SIX2, WT1 and PAX2) were epigenetically dysregulated in the WT blastema with aberrant DNA methylation at the promoter regions [113] and abnormal chromatin modifications [114]. These changes may lead to an enhanced activation and accumulation of early stem/progenitor cells that fail to undergo the normal kidney-specific differentiation program.

Pode-Shakked et al. reported that NCAM1, a marker for normal embryonic renal progenitors, is also a putative CSC marker in WT based on microarray data [115]. It was further found that NCAM1(+) cells showed the expression of a “WT-stem” signature gene set and clonogenicity. The group also performed serial transplantation of a NCAM1(+) cell fraction within WT into immunodeficient mice, demonstrating that NCAM1(+) cells give rise to new tumors as well as NCAM1(−) differentiated cells. Therefore, the NCAM1(+) population fits the criteria of CSCs, as they can self-renew and possess multipotency in vivo. Moreover, they demonstrated that targeting the human NCAM(+) cell fraction with an anti-NCAM antibody-drug conjugate resulted in loss of the WT CSCs, both in vitro and in vivo [115].

Shukrun et al. purified the CSC population in WT and revealed that NCAM1(+)ALDH1(+) cells had tumorigenic properties and exclusively localized within the blastemal compartment of the tumor, but that NCAM1(+)ALDH(−) or NCAM1(−)ALDH1(+) cells did not [116]. Surprisingly, they showed that ALDH1(+) WT CSCs are not the most primitive cell population in WT blastema, but correspond to more committed epithelial progenitors that have reduced expression of several renal progenitor markers including SIX2, OSR1, PAX2, CITED1, WT1, and SALL1 and relatively high expression of epithelial markers. Thus, it can be deduced that WT CSCs, which sustain tumor growth, can both dedifferentiate toward $\text{SIX2}^{\text{high}}$ blastemal cells and differentiate into mature epithelial structures.

More recently, Raved et al. used primary WT specimens from 24 patients and their medical records to demonstrate that the NCAM1(+) ALDH(+) CSC population significantly correlates with adverse clinicopathologic parameters and poorer prognosis; this underscores the role of the CSCs in disease progression and supports assessing for these markers for better risk stratification of WT [117].

Towards Clinical Applications and Practical Use

One of the remaining challenges in the directed differentiation of PSCs is the induction of mature collecting duct cells that include principal and intercalated cells. Additionally, PSC-derived embryonic kidney stromal progenitors have not been generated. Another challenge is that PSC-derived kidney organoids remain very immature. Possible solutions for the latter include vascularization and the addition of physical stimuli, such as blood or urinary flow and shear stress. To that end, organ-on-a-chip technology has already been used to mature human iPSC-derived nephron structures by incorporating a vascular network of endothelial cells [118].

To generate functional kidneys, human PSC-derived kidney tissues need to be connected to larger vessels, and the collecting ducts need to be attached to a renal pelvis and ureters. To generate larger kidneys, one promising approach is to utilize the embryonic niche of experimental animals including genetically manipulated pigs. Additionally, several studies have aimed to generate kidney organs by repopulating embryonic kidney and vascular progenitors inside scaffolds composed of the extracellular matrices that remain after kidney decellularization [119]. Another approach is to artificially generate 3D kidney tissues with a 3D printer [120].

To harness the beneficial paracrine effects of human iPSC-derived kidney progenitors against AKI, identifying the molecular nature and elucidating the protective mechanisms would facilitate more effective cell-based therapies. Also, human iPSC-based cell therapies that prevent the progression of CKD will hopefully be developed. Other research applications that could utilize PSC-derived kidney lineage cells include disease modeling, drug discovery, and toxicology screening. The discovery of novel drug compounds using PSC-based disease models is anticipated for intractable kidney disorders, such as autosomal dominant polycystic kidney disease (ADPKD) [5, 6].

As for fetal-derived cells, several therapeutic approaches can be envisioned. Fetal kidney-derived NCAM1(+) progenitors have already shown clinical benefit in a mouse model of CKD, demonstrating an ability to halt disease progression [43]. Considering the low immunogenicity of fetal kidney cells, it may be possible in the future to generate a tissue bank of cell for regenerative purposes. Furthermore, an equivalent population of fetal-like progenitors could be isolated from iPSC-derived kidney organoids, to generate autologous cells for treatment of kidney disease.

Similarly, although the adult kidney does not harbor bona fide multipotent stem cells, tubule-forming cells established from adult kidneys have already shown to successfully improve renal function in mouse models [92]; this paves the way towards potential patient-specific cell therapies or for the utilization of the bioproducts from these cells in a cell-free regimen. The identification of a synergistic effect of tubule- and vessel-forming cells for differentiation capacity in vivo also has the potential for beneficial effects on diseased kidneys, although the clinical benefits of this combination still need to be determined.

Regarding renal CSCs, a definitive selective marker for the isolation and targeting of these stem cells is still lacking. Future studies to identify appropriate selection markers and to understand the intracellular pathways that regulate renal CSC maintenance and differentiation should lead to new therapeutic strategies.

Conclusions

During the last decade, kidney regeneration research using human PSCs has substantially advanced. Directed differentiation protocols that induce human PSCs to differentiate into embryonic kidney progenitors, NPCs and UB cells, have been developed, and kidney organoids containing nephrons and collecting ducts can be generated from these progenitors. Development of novel therapeutic strategies using PSC-based technologies, such as cell therapies and the transplantation of reconstructed kidneys, is expected during the next decade.

Concomitantly, the ability to isolate and culture tubule-forming cells from both fetal and adult human kidneys is a promising milestone that could be harnessed in the future to develop cell-based, potentially autologous treatments. While treatments using adult cells would likely require a combination of cell types, the ongoing improvements in our understanding of kidney lineages and cell biology suggests that this is a possible, albeit yet still a challenging goal.

Both RCC and WT possess CSCs with high tumorigenic ability and resistance to chemotherapy and radiotherapy, thus providing a rationale for new therapies targeting these stem cell populations. The tumor formation model with WT CSCs could help us better understand kidney development and generate anticancer therapeutic strategies.

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Part II

Renal Physiology and Diagnostic Approaches

Assessment of Kidney Function in Children, Adolescents, and Young Adults

5

Guido Filler, Maria Ferris, and Jyothsna Gattineni

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Abstract

Kidney function undergoes developmental changes, including postnatal adaptation of nephron recruitment until approximately 18 months of age and glomerular hypertrophy thereafter. Accurate assessment of kidney function across post-conceptual age is important and needs to incorporate factors associated with the person (e.g., body habitus, growth, muscle mass, health status, illnesses, inflammation, and intra-personal variability), measurement technique (e.g., endogenous biomarkers, exogenous techniques, or imaging studies), and interpretation of measurements (e.g., equation used, correction factors, indexing to body surface area, or extracellular volume).

While nephron endowment would be the most important factor for an accurate clearance calculation of more than 60% of drugs processed by the kidney, this cannot be assessed directly. Effective renal plasma flow (ERPF) may be better to assess nephron endowment as it does not undergo autoregulation or hyperfiltration, in contrast to glomerular filtration. However, glomerular filtration rate (GFR) is still the best surrogate tool for kidney function assessment.

The gold standard to measure kidney function is inulin clearance; however, this is impractical. Measurement of GFR using either radiolabeled or cold exogeneous substances such as iohexol, iothalamate, etc. now serve as replacement for more accurate GFR determinations. In the clinical routine, endogenous markers are used, especially serum creatinine and cystatin C. Health care providers need to know when to use which endogenous biomarker(s).

In this chapter we will discuss historical aspects of kidney function measurement, exogenous and endogenous methods, and the technical aspects of assessment or interpretation in health and sickness, from the neonate to the transition-age periods.

Keywords

Clearance · Inulin · Para-Aminohippuric Acid · Effective renal plasma flow · Filtration Fraction · Nephron endowment · Creatinine · Cystatin C · Beta-trace Protein · IDMS traceability · Certified Reference Intervals · Reference Intervals · Race · Transition to adulthood

Abbreviations

AYA	Adolescents and young adults
CAKUT	Congenital anomalies of the kidneys and urinary tract
CKD	Chronic kidney disease
CysC	Cystatin C
DMSA	Dimercapto succinic acid
DTPA	Diethylenetriamine penta-acetic acid
EDTA	Ethylenediamine tetra-acetic acid
eGFR	Estimated glomerular filtration rate
ERPF	Effective renal plasma flow
GFR	Glomerular filtration rate
MAG3	Mercaptuacetyltriglycine
PAH	Paraaminohippuric acid

Summary and Key Points

- Kidney function cannot be assessed directly. Glomerular filtration rate (GFR) is currently the best surrogate of renal function.
- The gold standard for GFR measurement is inulin clearance, but it is impractical. However, there is good agreement with nuclear medicine

methods such as ^{99}Tc iohexol ^{99}Tc DTPA, ^{51}Cr EDTA, and, iothalamate. Iohexol and iothalamate can also be performed without radiolabeling.

- Creatinine, albeit a marker afflicted with many technical problems, remains the most commonly used endogenous biomarker of kidney function measurement.
- Cystatin C (CysC) is a superior biomarker of kidney function that can be used even at 1 day of life, but its availability is limited.
- Beta trace protein and beta-2 microglobulin may be future endogenous biomarkers used to assess kidney function.
- Exogenous methods to directly measure GFR are impractical in the daily clinical routine. Estimated GFR is more suitable and it should be based on serum creatinine and when available, CysC.
- Special populations require customized approaches for measurement and interpretation of kidney function.
- In the transition age, adolescents' eGFR calculation changes as soon as they turn 18 years of age, if reported by automatic calculation in the electronic health record.

Background

> In the kidneys are seated reasonings, and there dwells in them the faculty of discernment; they distinguish truth from falsehood, and judge what is base and what is noble. Saint Ephraem (ca 306–373 CE)

Kidney function cannot be measured directly [1]. One would have to calculate clearance which is the process of removing a marker (i.e., creatinine) over a given period of time, in this case through removal by the kidneys. It is important to measure kidney function as 60% of drugs are eliminated by the kidneys [2]. The process of glomerular filtration of plasma fluid has been known for over 170 years. In 1844, Ludwig proposed first the physico-mechanical formation of a protein-free ultrafiltrate by the renal glomeruli [3, 4]. The first biomarker of renal function was urea, which was initially isolated from urine in 1773 by the French chemist Hilaire-Marin Roeuelle. Urea preparation by the German chemist Friedrich Wöhler from ammonium cyanate in 1828 was the first generally accepted laboratory synthesis of a naturally occurring organic compound from inorganic materials [5]. Moller introduced urea clearance as a measure of kidney function in 1928–1930. He defined clearance as "the volume of blood that a 1 min's excretion of urine suffices to completely clear of urea" [6–9]. In the 1930s, during studies of the kidney tubules, investigators searched for a biomarker that would not be reabsorbed or secreted after introduction into the tubules. The ideal GFR marker must have stable concentration in the plasma, must be physiologically inert, cleared only by the kidney, and freely filtered at the glomerulus [10], without reabsorption or secretion by the tubules. Richards introduced inulin in 1934, because of its high molecular weight and its resistance to enzymes [11]. Its clearance was described by Shannon in 1935 [12]. Subsequently, inulin, which is only eliminated by glomerular filtration (without undergoing any tubular absorption or secretion), became the standard for measuring kidney function. Smith introduced the inulin clearance for renal function measurement in 1956 [4].

Kidney clearance describes the volume of plasma that is completely cleared of a substance by the kidneys per unit of time. The kidney clearance of a substance x (C_x) is calculated as:

$$C_x = U_x * V/P_x$$

where V is the urine flow rate (mL/min), U_x is the urine concentration of the substance x, and P_x is the plasma concentration of substance x. Typically, C_x is expressed in mL/min, and normalized to 1.73 m^2 body surface area (BSA) [1]. If a substance is freely permeable across the glomerular capillary and is not synthesized, transported, or metabolized by the kidney, C_x is equal to the GFR [13].

Today, we use measured GFR as the gold standard for assessing renal function [1]. As we will learn, inulin clearance has been replaced by more available exogenous substances such as ^{51}Cr-ethylenediamine tetra-acetic acid (EDTA), ^{99}Tc-diethylenetriamine penta-acetic acid (DTPA), ^{125}I-iothalamate, and iohexol. Most of these substances are labeled with a radioisotope; however, iohexol can be used hot or cold. While now serving as the new standard, these methods are impractical for daily clinical use, and endogenous markers are widely used, especially serum creatinine, albeit its many limitations.

Two important aspects have to be mentioned. Firstly, glomerular filtration only accounts for a fraction of the removal of toxins from the body, as most toxic substances are highly bound to plasma protein and require tubular secretion rather than glomerular filtration. Homer W. Smith clearly demonstrated that para-aminohippuric (PAH) acid was the most suitable agent for the evaluation of kidney plasma flow in both humans and dogs and introduced the PAH clearance which is still used in some centers today [14]. PAH clearance more closely reflects nephron endowment when compared to GFR [1]. Due to the autoregulation of the kidney, GFR can be maintained across a wider range of nephron endowment. Only when the filtration fraction is normal (the ratio of GFR/PAH clearance, which is normally about 16%) and there is no hyperfiltration, GFR will accurately reflect PAH clearance [15]. GFR may be inappropriately high due to glomerular hyperfiltration [15]. It is thought that glomerular hyperfiltration can be caused by afferent arteriolar vasodilation, as seen in patients with diabetes or after a high-protein meal, and/or by efferent arteriolar vasoconstriction owing to activation of the renin-angiotensin-aldosterone system, thus leading to glomerular hypertension [16]. Unfortunately, we do not have an endogenous biomarker of PAH clearance, even though it has a strong predictive value for long-term prognosis in hemolytic uremic syndrome [17].

Secondly, we take it for granted that kidney function should be indexed to body surface area (BSA). However, does this make sense when (a) gaining body weight increases BSA which would automatically increase your GFR and (b) the body fat is not participating in the renal clearance? Moreover, the biological principle of having a finite number of nephrons until 36 weeks of gestation and subsequently experiencing slow attrition of nephrons [18] does not match the life course of GFR. Bird questioned indexing GFR to BSA for some time and proposed to use the extracellular volume (ECV) instead [19, 20]. After birth, nephrons are slowly recruited until about 18 months [21] and from then on, the normalized GFR should gradually diminish due to nephron attrition, but only when indexing GFR to ECV does this happen [19, 20, 22]. Peters et al. published a robust formula for the calculation of ECV [23], but there is no consensus about indexing GFR to ECV rather than BSA.

The Importance of Measuring Kidney Function

Measuring kidney function guides clinical practice, drug dosing, and research. Identifying impaired kidney function is paramount as more than 60% of drugs are cleared by the kidney [2]. The preservation of kidney function is a widely important goal for managing acute and chronic kidney disease conditions. The progression of chronic kidney disease (CKD) is monitored by serial GFR assessment [24]. Assessment of kidney function is also important as a predictor of outcomes, for instance, in heart failure [25, 26]. Health providers must therefore be very familiar with the various methods of assessing kidney function, be aware of the strengths and limitations of the various techniques, and be especially mindful about technical aspects when using either endogenous or exogenous markers of kidney function [1, 27].

Current Gold Standard/Exogenous Methods

There are significant limitations to the inulin clearance, including limited availability and methodological aspects for the quantitation of inulin in the blood and urine samples with substantial inter- and intra-patient variability [1]. However, if inulin clearance is employed, we would recommend a bolus injection of 5000 mg of inulin per 1.73 m^2, with a maximum dose of 5000 mg. Inulin has to be infused at a constant rate, over 30 s. Extravasation must be avoided, as this would result in an overestimation of GFR. Sampling times must be 4 or more, with an ideal extension to 240 min. For the evaluation, a two-compartmental model using the concentrations at the actual time points must be utilized, ideally with NonMem or other appropriate pharmacokinetic software programs like WinNonLin [28]. It is unclear if any centers are still utilizing inulin clearance. There is only one supplier left in the world who provides Inutest®, and at a prohibitive cost.

In the 1970s and 1980s, inulin clearance studies were gradually replaced by nuclear medicine methods. These methods typically involve the injection of a radiolabeled pharmaceutical with features similar to inulin, although none of them have zero plasma protein binding, which would be essential for pure glomerular filtration measurement. Iohexol is not radiolabeled and has only 2% plasma protein binding, making this the preferred method [29]. The quantitation of the pharmaceutical through the simple nuclear medicine method of simply counting the disappearance of the isotope counts over time allowed for faster and more accurate measurement of GFR. The most commonly used exogenous markers of GFR are listed in Table 1. Each center will have experience with one particular method and will have particular reasons for choosing one method over the other. For instance, at the University Medical Centre Groningen, Netherlands, ^{125}I-iothalamate is used combined with ^{131}I-Hippuran for the simultaneous determination of ERPF [30]. ^{131}Iodine emits radiation in the form of medium energy gamma rays and mostly beta particles, whereas ^{125}Iodine emits soft gamma radiation with a maximum energy of 35 KeV so that both isotopes can be identified with different cameras.

Comparison of Exogenous GFR Methods, Technical Aspects

Iohexol is theoretically superior to other exogenous markers because of its low plasma protein binding: the higher the plasma protein binding, the larger the proportion of clearance contributed by tubular secretion, leading to the overestimation of GFR. In practice, however, the accuracy of iohexol GFR estimates demonstrate considerable scatter [42]. Clinical studies comparing inulin clearance with nuclear medicine scans favor ^{51}Cr EDTA, which showed the least bias and best agreement in both transplant and non-transplant patients [1, 32]. In a study of 40 renal transplant patients, the error was only between +2.2 to

Table 1 Commonly used exogenous markers of glomerular filtration rate (GFR)

GFR Compound	Places of use	Major features	Plasma protein binding
^{51}Cr-ethylenediamine tetra-acetic acid (EDTA) clearance [31]	Europe	Tight binding of chromium to pharmaceutical, radiation dose 0.074 MBq/kg to 3.7 MBq/kg. Can be combined with DMSA or MAG3 imaging study [32]. Not available in the USA and Canada. Beta-particles from ^{51}Cr can be carcinogenic.	12.2% [33]
^{99}Tc-diethylenetriamine penta-acetic acid (DTPA) [34]	Most of the world except Europe	Often used together with imaging, maximal dose 37 MBq, (i.e., higher than ^{51}Cr EDTA). If DTPA gamma camera images and relative function are required, the dose should be higher [32].	11.0% [33]
^{125}I-iothalamate [35–37]	USA	May allow for the simultaneous assessment of extracellular fluid volume and GFR [30]. Can be combined with ^{131}I-Hippuran for simultaneous determination of ERPF [30]. Up to 38% overestimation when compared to inulin in healthy subjects [38]. Used as subcutaneous injection in Montreal [39].	9.6% [33]
Cold iohexol [40, 41]	Scandinavia, North America	No radiation exposure, also has lowest plasma protein binding.	2.0% [29]

+2.8 mL/min/1.73 m^2 when comparing ^{51}Cr EDTA and inulin clearance [43]. ^{51}Cr EDTA is not available in the USA and Canada.

There are important technical aspects to be considered when employing exogenous methods. Perhaps the most important one is the extravasation of the pharmaceutical. Nuclear medicine methods allow for an assessment of extravasation by taking a gamma (or beta) camera picture of the injection site [1]. Samples must not be drawn before the infused substance reaches equilibrium with the entire extracellular volume [1]. Errors can also occur if the analysis method does not include a proper nonlinear two-compartmental model [1]. Many centers only employ two time-point measurements instead of three, which makes it impossible to reveal more than usual equilibration of the pharmaceutical between both compartments [1]. In Fig. 1, we show a typical 2-compartmental model of the isotope distribution. As seen from this figure, sampling should not start before 45 min. In patients with dyslipidemia, the time to equilibration can be much longer [44]. In patients with very low GFR, late samples have to be drawn [45]. In an unpublished survey among Canadian pediatric nephrology centers, the majority of centers employed only two sampling points and not three, which may result in significant overestimation of GFR.

Furthermore, most centers employ a single shot plasma clearance method, even though inulin clearance uses timed urine collections. The major disadvantage of plasma clearance is the duration of testing needed to calculate the clearance curve accurately, which can take up to 8 h [45]. As an alternative, urinary clearance techniques are another direct method that can be used to measure GFR, but these remain inconvenient and associated with errors around the timing of urine collections. We evaluated this in our center and found a significant difference when comparing plasma disappearance with a urinary method [46]. The mean (with standard deviation) plasma isotope GFR of 60.7 ± 24.9 ml/min/1.73 m^2 compared to urinary isotope GFR of 52.0 (28.0) ml/min/1.73 m^2 was statistically significant (p-value <0.001). The urinary clearance of ^{99m}Tc-DTPA was calculated using the urinary clearance equation:

$$GFR_{i_{Urine}} = \frac{U_i \times V_i}{(T_i - T_{i-1}) \times \exp\left[0.5 \times (\ln P_i + \ln P_{i-1})\right]}$$
$$\text{where } i = 1, 2, 3 \text{ (sample)}$$

$$\begin{aligned} GFR_{Urine} &= median(GFR_1, GFR_2, GFR_3) \\ &\quad \times \left(\frac{1.73}{BSA}\right) \quad \text{where } BSA \\ &= 0.007184 \\ &\quad \times \left(height^{0.725} \times weight^{0.425}\right) \end{aligned}$$

where U_i is the urinary concentration of ^{99m}Tc-DTPA adjusted for isotopic decay rate, V_i

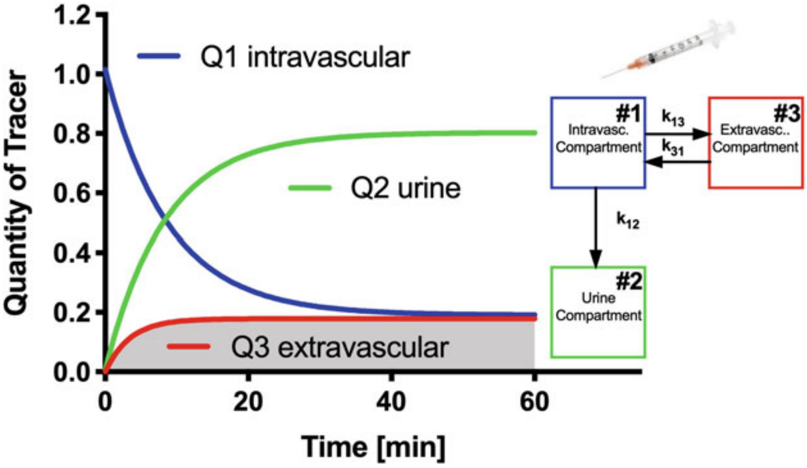

Fig. 1 Nonlinear 2-compartmental model of pharmaceutical disappearance after a single shot intravenous application of a GFR marker. Abbreviation: *Q* quantity

is the urinary volume, and P_i is the plasma concentration of ^{99m}Tc-DTPA adjusted for the isotopic decay rate, and T_i and T_{i-1} are the two time points during which the urine volume was collected [46]. Although plasma and urinary iGFRs were highly related ($R^2 = 0.86$), plasma isotope GFR increasingly overestimated urinary isotope GFRs at lower GFR levels [46]. However, urinary isotope GFR measurements are not very practical. Another problem is the timing of the sampling and the common practice of simply calculating the slope of the log-transformed isotope counts, rather than plugging in the exact counts with exact time points, in a two-compartmental pharmaceutical clearance program, such as WinNonLin or WinMem [1].

One study found that very few centers employed a measurement of GFR by utilizing a single subcutaneous injection of ^{125}I-iothalamate [47], where the correlation coefficient was quite reasonable ($r = 0.97$). However, no Bland & Altman analysis was performed to assess agreement and there were many outliers. The big advantage of this approach is the avoidance of extravasation, which may explain the relatively low intra-patient variation of repeated subcutaneous ^{125}I-iothalamate clearance (average maximum coefficient of variation was 7%) [47]. Given that proper nonlinear multi-compartmental models need to be utilized for accurate measurement of the isotope disappearance, it is understandable that not many centers utilized this approach.

Ideally, each pediatric nephrology provider should meet with their nuclear medicine department and review the often decade-old protocols to ensure that:

1. Images of the injection site are available to account for extravasation.
2. Sampling does not start too early (minimum 60 min and in nephrotic patients, possibly 90 min).
3. Three sampling points are utilized.
4. Two-compartmental nonlinear models with exact time points after injection are employed, rather than a simple calculation of the slope of the regression line of the log-transformed isotope counts.
5. The simple old Brøchner-Mortensen correction for the slope-intercept technique that is frequently used in old protocols is replaced [48].

The notable accuracy and reproducibility of GFR measurements will improve after the implementation of all of the points noted above. Providers should not be surprised if GFR results are 8–10% lower than previously noted after implementing the steps above, especially at lower levels of GFR.

Unfortunately, there is a paucity of meta-analyses of studies comparing inulin clearance with plasma and urine nuclear medicine GFR methods. We are only aware of one systematic review which concludes that there is at least moderately strong evidence suggesting that renal clearance of ^{51}Cr-EDTA or iothalamate and plasma clearance of ^{51}Cr-EDTA or iohexol are sufficiently accurate methods to measure GFR [49].

Fluorescent GFR Markers

Recently, two novel fluorescent markers that can provide accurate real time assessment of GFR have been studied in humans [50–52]. Each of both markers has been shown to be freely filtered by the glomerulus without tubular secretion or absorption. In addition, each of these markers do not undergo extrarenal metabolism, are not protein bound, or affected by medications. The study by Rizk et al. used a large 150-kD rhodamine derivative and small 5-kD fluorescein carboxymethylated dextrans which shows a linear correlation with measured iohexol GFR in 32 adults [50]. Like Inulin, 5-kD fluorescein carboxymethylated dextrans are carbohydrate polymers that are commercially available. Furthermore, the dextrans are neutral in charge like inulin; however, the carboxyl groups will likely cause an overall negative charge of the molecule [53]. It is important to note that the method proposed by Rizk allows for sufficient time for the equilibration of the exogenous substance between the compartments, which may explain the bias towards overestimation in the lower GFR range when using this method [54]. The other fluorescent detection system translates decline in transdermal fluorescence signal of the agent into GFR measurement. These methods are still considered experimental but hold promise for real time GFR assessment at the bedside in the near future.

Impractical Aspects of Exogenous GFR Measurement

Many centers routinely perform annual GFR measurements in their patients with CKD. As stated above, inulin clearance became impractical in the 1980s, and nuclear medicine methods or cold iohexol clearance replaced inulin clearance. Non-inulin methods to measure GFR have inherent challenges. From the institution's perspective, these are the challenges: The need for a technician or a nurse trained in the handling of radioisotopes is required for nuclear medicine scans. The need for the necessary equipment to measure the radioisotope and processes for safe disposal of these isotopes is essential. Both accurate bookkeeping and a special license to hold and use radioactive isotopes are required. Based on the frequency of the test being administered, the timely delivery of the radioisotope is important, as well as the appropriate usage of the radioisotope before its decay. To ensure that the patient is safe to receive the isotope, there is a need for a pregnancy test, and documentation of past allergic reactions. From the patient's stance, these are the following drawbacks: the need to be in the hospital for an extended period, large-bore IV access for frequent blood draws, administration of IV radioisotope, in some instances, the need for iodine prior to the procedure.

Endogenous Markers of Kidney Function

Serum Creatinine

Creatinine, 2-imido-5-keto-3-methyl-tetrahydroimidazole is the internal anhydride of creatine. Creatinine is derived from spontaneous nonenzymatic, nonreversible degradation of creatine and phosphocreatine in the muscle, and approximately 2% of muscle creatine is converted to creatinine daily. Many organs, including the kidney, liver, pancreas, and testes, contribute to creatine synthesis. Creatine is taken up by the muscle against its concentration gradient. In the muscle, the ratio of creatine:phosphocreatine is approximately 1:2. Creatinine in humans under physiological conditions is considered an inert substance with some exceptions. Creatinine is low molecular weight (113 Daltons), not protein-bound, freely filtered at the glomerulus, and secreted by the tubule [55].

Creatinine was first isolated by Chevreuk, a French chemist in 1832 from "beef-tea" and named it after the Greek word flesh [56]. Liebig in 1847 confirmed the presence of creatine in the muscle of animals and described the conversion of creatine to creatinine [57]. Colorimetric assay evaluating the color development between creatinine and alkaline picrate was first described by Jaffe in 1886 [58]. Folin in 1904 first described the colorimetric method of measuring creatinine by using the Duboscq colorimeter [59] and proposed to use urinary creatinine as a measure of completion of urine collection. This method was further modified to measure creatinine in urine and blood [60]. Popper, Mandel, and Meyer revised the Jaffe principle of measuring serum creatinine and obtained levels of 0.5–1 mg/dL while serum creatinine was 1–2 mg/dL using Folin's method [61]. One of the differences was the use of a colorimeter by Folin vs. the use of a photometer by Popper. Jaffe's reaction recipe has been modified by many investigators over the years and it became apparent that non-creatinine substances in the serum would react in the presence of picric acid, and these were subsequently labelled as non-creatinine chromogens. To decrease the interference of non-creatinine chromogens, Miller and Dubos used two bacterial enzymes to decompose creatinine in blood and used the Jaffe reaction to measure total creatinine equivalent before and after the addition of these enzymes [62, 63]. Despite the advent of specific enzymatic creatinine assays, Jaffe-based creatinine assays remain widely used across the world due to its low cost and simplicity.

Jolliffe and Smith in 1931 described creatinine clearance for the first time although urea clearance was described a few years prior [64]. Although some authors in the 1920s questioned the presence of any appreciable amount of endogenous creatinine and that creatinine was synthesized in the kidney, studies in the 1930s provided evidence that creatinine was indeed present in the circulation and could be measured. Rehberg in 1926 spearheaded the concept of using exogenous creatinine to measure GFR, and while exogenous creatinine clearance was higher than true GFR, he pioneered the concept of giving an exogenous substance to study renal dynamics [65]. Further studies in humans by Shannon and Miller and Winkler demonstrated that exogenous creatinine clearance was higher than GFR measurement using inulin [12, 66, 67]. However, Miller and Winkler also noted that endogenous creatinine clearance in healthy individuals equaled to inulin clearance, and the enzymatic method for creatinine measurement was used as developed by Miller and Dubos [62, 63]. These authors also noted that endogenous creatinine clearance was higher than inulin clearance in patients with chronic glomerulonephritis. An additional study in humans with kidney disease (secondary to heart disease or intrinsic kidney disease) employed two different creatinine measurements: total creatinine chromogen and specific creatinine levels, and demonstrated that endogenous creatinine clearance is not a satisfactory marker as it overestimated GFR by inulin clearance [68, 69].

Popper and Mandel proposed the use of serum creatinine in 1937 [61], which remains to be the most widely used marker for GFR estimation in spite of the following shortcomings and technical issues:

- **Tubular secretion:** One of the reasons creatinine is not an ideal marker for estimation of GFR is its tubular secretion [1, 66, 69–72]. Specifically, in CKD, as the tubular secretion of creatinine is maintained, the proportion of secreted creatinine increases in relation to the filtered creatinine resulting in GFR overestimation. Thus, the contribution of secreted creatinine to overestimation of GFR is important and worsens even more in CKD [72]. Tubular secretion of creatinine divided by inulin clearance increases progressively from 0.16 in adult patients with normal GFR, to 0.92 in patients with GFR less than 40 ml/min [70]. In addition to secretion, creatinine may undergo reabsorption as well [73].
- **Blockade with H2 antagonists and other medications:** Blockade with H2 antagonists revealed promising results that may overcome the significant problem of tubular secretion of serum creatinine [74, 75]. The use of cimetidine protocols in children remains scarce [76]. In addition to H2 blockers, trimethoprim and fenofibrate inhibit tubular secretion of creatinine resulting in higher serum creatinine without a change in GFR.
- **Dependency on muscle mass:** The source of creatinine is creatine, which is related to the muscle mass of the individual. Males have higher muscle mass than females and children have a lower muscle mass than adults [77–79]. Thus, the normative values for creatinine change as children get older and their muscle mass increases. Cancer patients have cachexia-related muscle wasting even if they have obesity, precluding steady state measurements.
- **Dependency on nutrition:** Dietary intake of protein also plays an important role in changes in serum and urine creatinine without necessarily changes in GFR. Uncooked meat contains creatine and increasing meat intake increases creatine, and thus, increasing creatinine production and excretion. Additionally, cooking meat converts creatine to creatinine, which is then easily absorbed from the gastrointestinal tract which in turn increases serum creatinine and its excretion [80, 82].
- **Non-renal clearance:** Extra-renal clearance of creatinine should also be considered when using creatinine as a marker of GFR. An extra-renal clearance of 1.7–2 ml/min [81, 83] by the gastrointestinal tract has been described, which is negligible in a healthy adult with a creatinine clearance of ~120 ml/min, but in a

patient with CKD who has a creatinine clearance of 3–5 ml/min, this becomes relevant.

- **Issues with the analytical method:** There used to be considerable variability in the reference range for serum creatinine based on the method used for its determination [84, 85]. Proficiency testing surveys published periodically by the College of American Pathologists evaluated the variability in creatinine measurement among various methods used by different institutions across the USA, and demonstrated substantial variability [86]. Fortunately, the traceability of the creatinine measurements to higher-order reference methods (isotope dilution-mass spectrometry -IDMS- reference method) improved the accuracy of creatinine measurements in recent years [87]. Pediatric health care providers must be aware of the method used for serum creatinine measurements. Despite IDMS traceability, the original Jaffe method is influenced by chromogens such as bilirubin, ketones, some proteins, and drugs like cephalosporines [88, 89]. We are aware of the following methods for the measurement of creatinine: Jaffe, compensated Jaffe, enzymatic liquid, and dry chemistry methods. In 2015, after comparing all of these methods in 26 routine instruments representing 13 different creatinine methods and finding that CKD stage classification differed in 47% of cases, Hoste et al. wrote: “Although most creatinine assays claim to be traceable to the gold standard (ID-GC/MS), large inter-assay differences still exist. The inaccuracy in the lower concentration range is of particular concern and may lead to clinical misinterpretation when the creatinine-based eGFR of the patient is used for CKD staging. Further research to improve harmonization between methods is required.” [90].
- **Creatinine clearance with timed urine sampling:** Similar to what we wrote about urinary isotope clearance studies, creatinine clearance determinations involving timed urine collections (i.e., 24-h creatinine clearance) [69, 91] can improve the accuracy of GFR estimation. However, timed urine collections are highly inaccurate in children, difficult to perform, time consuming, and impractical [91].

Small Molecular Weight Proteins as Markers of GFR

In the search for novel endogenous GFR biomarkers that are not affected by muscle mass and diet, Jung and others proposed small molecular mass proteins as markers of GFR since they are almost freely filtered through the normal glomerular basement membrane [88, 121, 157]. In normal kidney function, these small molecular weight proteins are almost completely reabsorbed and degraded by the proximal tubular cells through pinocytosis. Several proteins have been considered good candidates for the measurement of kidney function, such as cystatin C (CysC), beta-trace protein (BTP) (analyzed in children), and beta-2 microglobulin (B2M) [93]. CysC is the best studied small molecular weight protein as a surrogate GFR marker and its superiority for the detection of mildly impaired GFR was proven in meta-analyses [94, 95]. However, all plasma or serum proteins can be influenced by circumstances in which protein synthesis is increased, as seen in hyperthyroidism [96] or decreased, as seen in prolonged low-protein diets [97]. This interaction between overall upregulated or downregulated protein synthesis is important. The clinician must be aware that such conditions can influence the serum or plasma concentration of small molecular weight proteins being used as markers of GFR. Another potential problem is hypertriglyceridemia, which can affect the concentrations of these biomarkers, as shown for CysC, where triglycerides clearly affect the CysC recovery [98]. Nonetheless, CysC, specifically, has substantially improved our ability to estimate GFR. B2M is an acute phase protein, associated with inflammation [99]. However, a recent study in unborn children with obstructive uropathy has demonstrated good results for the measurement of GFR in the fetus [100].

Cystatin C (CysC)

CysC is a low-molecular-weight protein (13.3 kDa) produced in all human cells that have a nucleus [101]. Like creatinine, CysC is filtered freely through the glomeruli, but unlike this endogenous marker, CysC is less affected by non-renal factors. The features of CysC have been described in detail elsewhere [55, 93, 102–104]. Serum levels of CysC do not correlate with any pathophysiological state other than those affecting the GFR in large patient cohorts, in keeping with a stable secretion of this low-molecular protein from most human tissues and no non-renal elimination [104]. However, very large doses of glucocorticoids have been described to increase the production of CysC [105, 106], whereas low and medium doses of glucocorticoids do not seem to alter its production [107]. Also, as discussed, thyroid dysfunction may affect CysC levels [108], although the findings are inconsistent, as in some of our recent studies, we found no association with thyroid function markers [109, 110].

The reference values for CysC obtained in a carefully selected French mostly Caucasian population are 0.75 ± 0.09 mg/L for children aged 4–19 years; 0.74 ± 0.10 mg/L for males and 0.65 ± 0.09 mg/L for females aged 20–59 years, and 0.83 ± 0.10 mg/L for individuals older than 60 years of age [111]. In the first year of life, renal function matures physiologically. Accordingly, much higher CysC values up to 2.8 mg/L were found at birth. The rapid decline after birth reflects maturation of kidney function [113–115]. After birth, CysC concentrations fall, similar to serum creatinine, whereas the estimated GFR using the Filler formula [115] slowly increases until 18 months of life [18]. New reference intervals with more detailed age distributions of CysC from central Europe have recently been published on 985 healthy people older than 25 years [116].

After these encouraging findings, additional studies suggested that the reciprocal of CysC correlates better with a gold standard GFR measurement than the reciprocal of serum creatinine [118–120]. CysC, as a marker of GFR, was found to be independent of body composition [121–123]. Current automated and rapid particle-enhanced immunoturbidimetric and immunonephelometric methods (more precise than the original radio-immuno- or enzyme-linked immunosorbent assays) [123, 124] have allowed large-scale use of serum cystatin C as a clinically useful GFR marker [124].

The diagnostic performance of CysC was compared to serum creatinine in 2002 with a meta-analysis of 46 studies, in both adults and children [125]. Pooled data analysis of 3703 individuals compared correlation coefficients between GFR and the reciprocals of serum creatinine and CysC reported significantly better correlations for CysC (mean $r = 0.816$ [95% confidence interval: 0.804–0.826]) versus creatinine (mean $r = 0.742$ [95% confidence interval: 0.726–0.758]). Receiver operating characteristic (ROC) plots were available for a pooled sample of 997 individuals, again showing a significantly better area under the [ROC] curve (mean = 0.926 [95% confidence interval: 0.892–0.960] for CysC versus mean $r = 0.837$ [95% confidence interval: 0.796–0.878]) for creatinine. This meta-analysis of cross-sectional data suggests that CysC is superior to serum creatinine for the detection of impaired GFR. Another meta-analysis of 24 studies ($n = 2007$) also confirmed that the diagnostic accuracy favored CysC [95], although, in this more recent study, the diagnostic odds ratios started to overlap, most probably due to the IDMS testing of serum creatinine.

It should be highlighted that the lack of standardization of CysC assays and internationally certified reference materials lead to similar problems as with serum creatinine, unless IDMS traceability is considered [87]. Results obtained with the two main assays that are commercially available, namely, the DAKO kit (turbidimetric, PETIA) and the Siemens Healthcare assay (nephelometric, PENIA), can be quite different, as demonstrated with reference intervals for healthy term and preterm infants. The results with the PENIA method in our study [126] were significantly lower than those reported by Harmoinen [127]. Similar problems occurred when the results of the CKiD study were re-analyzed using PENIA, which rendered substantially better results [128]. Grubb reported the first certified standardized reference material for cystatin C [129] and most commercial suppliers of CysC are now using these certified reference intervals. Unfortunately, the availability of CysC is still lacking in many parts of the world. This is despite the fact that for the best accuracy of estimation of GFR from endogenous biomarkers, a combination of creatinine and CysC is recommended, both for children [128] and adults [130]. There are problems with knowledge translation. In London, Ontario, it took years and intense collaboration with the clinical biochemists to introduce a cost-effective solution on the existing multi-analyzer platform with a turn-around time of 1 h [131].

Serum Cystatin C to Creatinine Ratio

It has been proposed to use a ratio of CysC and creatinine as a marker of disease. The concept evolved after noticing significantly elevated cystatin C to creatinine ratios during near-term pregnancy. There are conditions, such as the third trimester of pregnancy, where CysC increases but serum creatinine remains normal or even decreases due to hyperfiltration. Furthermore, CysC is heavily expressed in the placenta towards the end of pregnancy for unknown reasons. Investigators have proposed that serum CysC C/creatine ratio as a good estimate of kidney function in Korean children with CKD [132]. Others proposed the GFR CysC/GFR creatinine as a better estimate for children with CKD [133]. Children with solitary functioning kidneys have hyperfiltration and are at risk for CKD. The KIMONO Study determined the precision of eGFR formulas (two for creatinine and two for CysC) in children with a single kidney. These investigators recommended the combination of serum CysC/creatinine-based eGFR for children with solitary kidneys [134]. Age- and sex-specific reference intervals for serum CysC/creatinine ratio in healthy 0 to 18-year-old Chinese children have also been determined, noting that this ratio decreased with age and was higher in girls [135]. In this context, a concept of "shrunken pore syndrome" was developed [136], mainly characterized by a higher low molecular protein concentration compared to the level of GFR and creatinine. We are unaware of any histopathological confirmation of this "shrunken pore syndrome."

Beta Trace Protein (BTP)

Another potential surrogate marker for GFR measurement is BTP [138–140], a low molecular weight protein (MW 20-31 kDa) also known as prostaglandin D2 synthase (PGDS; prostaglandin H2 D-isomerase [EC 5.3.99.2]). BTP has traditionally been used as a marker for cerebrospinal fluid (CSF) leakage, as it represents approximately 3% of the proteins in the CSF [140, 141]. The availability of serum BTP for the measurement of eGFR is limited [142–144]. Although both brain and serum BTP are composed of an identical amino acid sequence, variations in molecular size can be attributed to differences in oligosaccharide structure and N-glycosylation. Serum and urine BTP have longer sugar chains and higher sialylation than BTP in the CSF [144].

Beta trace protein shares many of the features of CysC as a marker of GFR [93]. Priem et al. were the first to describe BTP as a new marker of GFR in the creatinine-blind range in 1999 [145]. We have previously described the history, indications, benefits, and limitations of BTP as a marker of GFR [141]. Some possible advantages of BTP over CysC are that it does not have a significant relationship with c-reactive protein (a marker of inflammation), is unaffected by body composition, adequately reflects GFR during the third trimester, and its concentration is not impacted by thyroid function [146]. In children and adolescents from London, Ontario, the reciprocal of BTP correlates strongly with both ^{99}Tc DTPA and ^{51}Cr EDTA measurements of kidney function (Fig. 2). Donadio had similar results with an excellent correlation coefficient of $r = 0.924$ for BTP [147]. Bökenkamp also demonstrated a good correlation between BTP and GFR. In fact, his study suggested that BTP has a better diagnostic sensitivity than CysC when GFR is mildly impaired [148]. Unlike CysC, which is strongly positively charged,

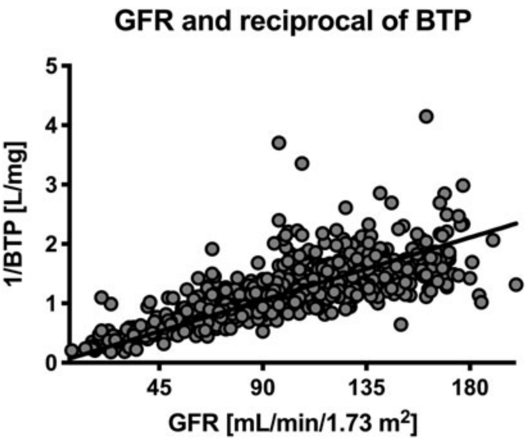

Fig. 2 The relationship of 1/BTP measured using the Siemens Healthcare BTP nephelometry assay in 825 Canadian children and adolescents aged 2–18 years of life with GFR measured using ^{99m}TcDTPA. Pearson r was 0.7102, 95% confidence interval 0.6745 to 0.7426, $p < 0.0001$

BTP has an isoelectric point of 5.5 or lower [149, 150]. BTP appears to be a more favorable marker in pregnancy [151] and in newborns [152]. Pediatric reference intervals have been published that include newborns and infants [153]. Formulae for the estimation of GFR based on BTP have been developed and validated for adults [143] and for children [138]. Leslie Inker published a study from the CKD-EPI dataset, using BTP and beta-2 microglobulin, which unlike creatinine or CysC did not show any differences due to sex [154]. However, this formula cannot be used for children [155].

Unfortunately, the availability of BTP is limited and the cost is quite high. Unlike for CysC and creatinine, there is no higher order reference material available to standardize the measurement. The two main assays by Cayman Chemicals (an ELISA method) and Siemens Healthcare (a particle-enhanced nephelometric immunoassay using polyclonal rabbit antibodies against human urinary BTP), use different analytical techniques and different antibodies, which may recognize different glycoprotein epitopes and therefore, may not bind to all BTP isoforms.

Beta-2 Microglobulin

Beta-2 microglobulin (B2M) is a low molecular weight protein (MW 11.8 kDa) and part of a class of major histocompatibility antigens expressed on the cell membranes of nucleated cells. B2M is freely filtered at the glomerulus, reabsorbed and catabolized in the proximal tubule by endocytosis [156, 157]. B2M is released into the systemic circulation during cell turnover and its volume of distribution is considered to be in the extracellular space. Bernier, Cohen, and Conrad were the first investigators who noted that serum levels of B2M were up to 150 times higher in patients with kidney disease of varying etiologies than those with normal kidney function [158]. In addition, these investigators found that serum B2M correlated with creatinine in patients with kidney failure [158]. Using rat animal models, B2M was injected intravenously in normal and ureter ligated rats and no B2M appeared in the urine while serum concentrations decreased promptly, indicating that B2M is catabolized in the kidney [159]. However, in the anephric rat model, the serum half-life of B2M was prolonged [159].

Viberti and his colleagues measured serum B2M, creatinine, and ^{51}Cr-EDTA GFR in patients with long-standing diabetes and noted a significant negative correlation between B2M and creatinine with measured GFR, respectively. B2M was always above the normal range when the GFR was <80 ml/min/1.73m^2, while serum creatinine was within the normal range in two patients, even when the GFR was ~65 ml/min/1.73m^2. Additionally, creatinine showed a diurnal variation, while B2M levels remained unchanged during the day, and was independent of food intake [160]. Jung and his colleagues studied different low molecular weight proteins and creatinine, correlating their levels to measured GFR by ^{99m}DTPA [161]. Although ribonuclease and B2M were more sensitive than creatinine in detecting low GFR, the large scatter of GFR confidence interval limits which corresponded to the upper reference limits of B2M and ribonuclease, made them less acceptable for GFR prediction [92, 161]. Nonetheless, B2M appears to be a good GFR estimator in unborn children with congenital obstructive uropathy [162].

B2M can be measured in urine and serum using various commercially available methods including particle-enhanced turbidimetric immunoassay (PETIA) [163], particle enhanced nephelometric immunoassay (PENIA) [164], or enzyme-linked immunosorbent assay [165]. The test results can vary, depending on the assay used. Despite reference materials from the World Health Organization (WHO) (1985) and the Institute of Reference Materials and Measurements (2015), commercial laboratories do not always follow these standards, resulting in variable results [166].

Increased B2M levels can be seen in infections, liver disease, lymphoproliferative disorders, malignancy, and inflammation [169–172]. Other non-GFR determinants associated with increased B2M levels include higher body mass index (BMI), hypertension, higher c-Reactive Protein, and lower HDL cholesterol. Unlike creatinine, B2M levels are less affected by age, sex, and race, but they are affected by smoking, and urinary protein excretion [171]. Corticosteroids decrease B2M [172]. Therefore, all of these factors should be taken into consideration when using B2M in calculating GFR, as they can cause bias and errors in GFR estimation.

Traditionally, elevation of B2M in CKD has been attributed to decreased filtration and decreased catabolism by the proximal tubule. However, recently, the role of uremic toxins in decreased binding of B2M to MHC molecules on the cell surface, resulting in increased shedding of B2M, is being postulated [173].

Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) investigators pooled the date from three large studies in adults with CKD where GFR was measured using urinary iothalamate [156, 176–178]. The goal was to develop equations using BTP, B2M alone, or in combination and to compare them to CKD-EPI creatinine and CysC-based equations. These equations were validated in the participants from these three studies. Some non-GFR determinants varied between CysC, creatinine, BTP, and B2M, while some were common. The combined B2M-BTP equation did not perform any better than the CysC or creatinine equation, and it was less accurate than the combined CysC-creatinine equation.

In children with various kidney diseases, these newer equations employing BTP and B2M were applied using post-hoc analysis of previously published data by Filler and collaborators. Although all the three newer GFR equations correlated significantly with the absolute GFR (not corrected for BSA), the confidence interval was large and unacceptable for the combined B2M-BTP equation. Thus, at this time, the combined B2M-BTP equation cannot reliably be used in children [155]. B2M has been measured in cord blood and can be used to estimate kidney function in fetuses with severe obstructive uropathy [100, 177].

Based on these studies, combining multiple endogenous markers of GFR with different non-GFR determinants, could potentially help in developing GFR equation(s) with minimal bias and increased precision; accounting for renal function level, race, sex, body surface area, and BMI, among other variables. It is also possible that with future studies different formulae will be used in different populations and health conditions.

Ferrocyanide

Ferrocyanide clearance is equal to the measured GFR in the dog, but in men it is lower. Miller and Winkler noted that ferrocyanide was reabsorbed by the tubules and its excretion did not provide accurate measurements of GFR in humans compared to dogs [178]. While several researchers explored this biomarker in other species, such as the cat, it has not been developed further as a GFR biomarker.

N,N,N-trimethyl-L-alanyl-L-proline betaine (TMAP)

This endogenous biomarker recently described as metabolite x17299 N,N,N-trimethyl-L-alanyl-L-proline betaine (TMAP) [179], belongs to the class of organic compounds known as dipeptides.

Like inulin, it is not charged. Its Monoisotopic Molecular Weight is 228.14 g/mol. These are organic compounds containing a sequence of exactly two alpha-amino acids joined by a peptide bond. Using untargeted metabolomics, Bradley Urquhart and his group recently identified TMAP as a new small molecule that appears to be a promising endogenous marker for the estimation of GFR [180]. This biomarker outperformed serum creatinine in over 400 non-dialysis CKD patients significantly, with an area under the receiver operated characteristics (AUROC) plot of 0.815; compared to an AUROC of 0.745 for creatinine. In dialysis patients, it demonstrated a stunning AUROC of 0.993 [180]. Currently, efforts to improve these methods are underway to validate this biomarker. Based on preliminary unpublished results, this novel biomarker has an excellent potential to estimate kidney function, which may not be afflicted with any of the setbacks of creatinine or low molecular weight proteins.

Formulae for the Estimation of GFR

A large number of formulae have been proposed for the estimation of GFR. Still, the Schwartz bedside formula [181] remains the most widely used formula. Today, we know that the combination of CysC and creatinine plus/minus urea, yields much more robust estimations of GFR. Table 2 lists commonly used GFR equations, of which the improved 2012 Schwartz formula using the combination of creatinine, CysC, and urea, probably performs best in children [128]. However, this formula requires additional information, such as height, which is often inaccessible to the laboratory information system. As a consequence, in London, Ontario, the old Filler formula [115] is used for the estimation of GFR in the clinical routine. To the best our knowledge, this formula remains the only formula validated for both adults and children and for GFR levels in the hyperfiltration range.

It should be highlighted that each formula performs best in the range where it was generated [182]. For instance, the CKD-EPI formula was generated from almost 4000 gold-standard nuclear GFR measurements; however, less than 1% of these were generated in young adults under 30 years of age. This may be the reason for the poor convergence of the 2012 Schwartz formula and CKD-EPI [183]. In that study of 18–30-year-olds, the 2012 Schwartz formula consistently estimated lower GFR values than the CKD-EPI formula, and they only converge at age 30 [183]. Selistre proposed to use pediatric formula for adolescents and young adults (AYA) up to 30 years of age [184].

Kidney Function Measurement and Interpretation in Special Health Conditions

As noted above, certain populations require special considerations for kidney function measurement and interpretation. In spina bifida and muscular dystrophy, only CysC should be used to measure kidney function. The abnormal muscle mass in both conditions always leads to an overestimation of GFR if creatinine is used. The Zappitelli [186] or Filler formula [115] are useful in these conditions [27]. Young athletes taking creatinine supplements would also benefit from a CysC-only approach [190]. In contrast, eGFR estimation using small molecular weight proteins should be avoided in patients with increased protein production such as hyperthyroidism [27]. We recommend the educational resource about GFR measurement for special populations in *Pediatric Nephrology* for a thorough review of dietary effects, inflammation, protein catabolism, altered muscle mass, sex, and conditions such as wasting conditions [27]. For some conditions, such as cystic fibrosis and chronic pseudomonas infections, only nuclear medicine methods may be reliable [191].

Age-Related Issues of Kidney Function Measurement

Neonates In pre-terms, renal development is interrupted, affecting nephron endowment [192]. Measuring serum creatinine in the first

Table 2 Commonly used GFR equations

Equation name	Equation
Equations with serum CysC and without serum creatinine or urea	
1. **Bökenkamp** [117].	GFR (mL/min/1.73 m^2) = 137/serum CysC – 20.4
2. **Filler** [115].	GFR (mL/min/1.73 m^2) = 10^(1.962+ (1.123 * LOG (1/serum CysC))
3. **Grubb** [185].	GFR (mL/min/1.73 m^2) = 84.69 * serum CysC $^{-1.68}$ x 1.384 for age < 14 y
4. **Zappitelli (CysC)** [186].	GFR (mL/min/1.73 m^2) = 75.94/[serum CysC 1.17] if renal transplant, x 1.2
5. **Schwartz improved 2012 (CysC only)** [128].	GFR (mL/min/1.73m^2) = (40.9 ± 0.3)*[1.8/CysC (mg/L)]$^{(0.931 \pm 0.020)}$
6. **Grubb standardized material 2014** [187].	GFR (mL/min/1.73 m^2) = 130*CysC$^{-1.069}$*age[years]$^{0.117}$–7
Equations with serum creatinine and without serum CysC or urea	
7. **Schwartz improved 2012 (Cr only)** [128].	GFR (mL/min/1.73m^2) = (42.3 ± 0.3)*((height (m)/SCr (mg/dL)))$^{(0.780 \pm 0.016)}$
8. **Pottel full age Spectrum formula** [188].	GFR (mL/min/1.73m^2) = 107.3/(SCr/Q) for ages 2–40 years GFR (mL/min/1.73m^2) = 107.3/(SCr/Q)*0.988$^{(Age-40)}$ for ages >40 years; where Q-values are the mean or median SCr value for age-sex-specific healthy populations.
Equations with serum urea and without serum CysC or creatinine	
9. **Schwartz improved 2012 (urea only)** [128].	GFR (mL/min/1.73m^2) = (41.0 ± 0.5)*[30/BUN (mg/dL)]$^{(0.613 \pm 0.024)}$
Equations with serum CysC and serum creatinine	
10. **Bouvet** [189]	[(SCr (μM)/96) $^{(-0.35\ (+/-0.20))}$]*[(CysC (mg/L)/1.2$^{(-0.56\ (\pm0.19))}$]*[(body weight (kg)/45)$^{(0.30\ (\pm0.17))}$]*[age (years)/14)$^{(0.40\ (\pm0.16))}$]
11. **Schwartz improved 2012** [128] **(CysC + Cr).**	GFR (mL/min/1.73m^2) = (41.6 ± 0.3)*((height (m)/Scr (mg/dL)))$^{(0.443 \pm 0.026)}$ *[1.8/CysC (mg/L)]$^{(0.479 \pm 0.031)}$
12. **Zappitelli (CysC + Cr)** [186].	GFR (mL/min/1.73 m^2) = (507.76*$e^{0.003*height}$)/ (CysC$^{0.635}$*SCr$^{0.547}$ [μmol/L]) If renal transplant, x1.165 If spina bifida, x(SCr$^{0.925}$ [μmol/L])/40.45
Equations with serum creatinine and serum urea	
13. **Schwartz improved 2012 (Cr + urea)** [128].	GFR (mL/min/1.73m^2) = (41.9 ± 0.3)* [(height (m)/Scr (mg/dL))]$^{(0.662 \pm 0.021)}$* [30/BUN (mg/dL)]$^{(0.171 \pm 0.021)}$
Equations with serum CysC and serum urea	
14. **Schwartz improved 2012 (CysC + urea)** [128].	GFR (mL/min/1.73m^2) = (40.8 ± 0.3)*[1.8/CysC (mg/L)]$^{(0.796 \pm 0.027)}$ * [30/BUN (mg/dL)]$^{(0.157 \pm 0.022)}$
Equations with serum CysC, serum creatinine, and serum urea	
15. **Schwartz improved 2012 (Cr + CysC + urea)** [128] .	GFR (mL/min/1.73m^2) = (41.5 ± 0.3)*((height (m)/Scr (mg/dL)))$^{(0.417 \pm 0.026)}$ *[1.8/CysC (mg/L)]$^{(0.431 \pm 0.032)}$ *[30/BUN (mg/dL)]$^{(0.088 \pm 0.019)}$

3 days of life reflects maternal levels in all neonates, regardless of gestational age [114]. Because of this shortcoming, CysC [152, 193] or beta-trace protein [194] offer more accurate estimates of neonatal kidney function. Another indirect measure of kidney function in this age group includes renal length by ultrasound images. In the pre-term, nephron endowment is compromised [18], and their muscle mass is decreased so their creatinine levels are lower. Full-term healthy neonates will begin to reflect recruit nephrons and serum creatinine will be reflective of actual levels by day three of life. If the neonate was exposed to adverse circumstances in–utero, such as maternal malnutrition (as in the Dutch cohort that formed the basis for the Barker Hypothesis/thrifty phenotype), medications (i.e., angiotensin-converting enzyme inhibitors), alcohol, and other substances, then their baseline level of renal function may not be accurate or represent steady-state levels.

Children and Adolescents Ages 1–18 Years The eGFR in this age group has been calculated by using the modified Schwartz formula, which multiplies the height in centimeters times a constant (0.43), divided by serum creatinine. The results reflects the GFR normalized to 1.73 m^2 [195]. The modified Schwartz formula corrects by surface area, but it does not correct for sex or race [195]. While this formula was developed from data of children with chronic kidney disease enrolled in the North American Chronic Kidney Disease in Children (CKiD) Study [196], it has also been used for healthy children and adolescents.

Transition-Age Period With the advent of electronic health records, young adults 18 years and older receive an automated creatinine-based eGFR calculation, based on the CKD-EPI formula. Unlike in their childhood and adolescence, 18-year-old individuals suddenly receive an eGFR calculation accounting for sex, race, height, or weight, and this may result in abrupt changes in renal function estimation [130]. With the CKD-EPI equation, patients may be subject to significant overestimation of eGFR, particularly Black patients.

In the USA, we performed a single-center study of longitudinal creatinine measurements in 18–30-year-old patients with CKD and determined that the 2012 Schwartz and CKD-EPI equations, exhibit poor agreement during this age period, particularly in males [183]. Concordance improved steadily as patients aged, and convergence occurred at age 30, but these differences were less dramatic for females. Our findings suggest that switching to the CKD-EPI equation after age 18, may not be an appropriate method for estimating renal function in AYAs with CKD [183]. As previously stated, Selistre et al. proposed to use the pediatric formula for adolescents and young adults [184]. Vice versa, adult formula such as the Cockcroft Gault formula, cannot be used in children and adolescents [197].

All Ages In cross-sectional samples from Europe and the USA, 6870 healthy Caucasian and those with kidney disease, ages 1–70 years (735 children), helped develop the “full age spectrum” equation, using isotope/tracer-measured GFR [188]. For CysC, only the old Filler formula appears to be validated for all ages and also for all ranges of GFR including hyperfiltration [115]. The CysC formula for GFR estimation works well for children [182]. The adult CKD-EPI formula came from three large studies in three continents combined, but there were few young adults included. A similar international multi-center approach is needed for all age groups as proposed by Björk et al. [198].

Race-Related Correction in Kidney Function

Race and ethnicity are sociological constructs, and while these terms have been used to describe health outcomes and disparities, they have no biological role. In fact, based on genomic studies, there are no biological differences by race. The inclusion of race in eGFR equations is being re-examined, as it does not reflect the correct level of kidney function among Black people. In adults, the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation includes a multiplication factor for race and Black patients had a 16% higher mean measured GFR when compared to matched sex, age, and serum creatinine of non-Black patients [199]. Similar findings were described using the MDRD equation [174]. Among preadolescents and adolescents, Martin reported that Black individuals excreted greater amounts of creatinine [200].

Using a multiplication factor for Black patients causes delays in referrals to nephrology care, in enlisting on transplant waiting lists, and it may affect drug dosing. Furthermore, there is no guidance about people with mixed race. On the other hand, removing the race factor could result in overdiagnosis of CKD or ESKD, and potentially lead to errors in drug dosing. In some Asian countries, other race-specific equations for eGFR among adults have been used [201]. Also, pediatric formulas may work better in a given setting. The new Schwartz equation was developed in a large USA population of patients with CKD with great predominance of patients with obstructive uropathy. This formula had poor performance in healthy Japanese children, particularly after 14 years of age [202]. Finally, race is not recorded in some jurisdictions. For instance, in Ontario, Canada, hospitals and large provincial healthcare databases do not record race in the medical record [203]. Some formulas using small molecular weight biomarkers such as CysC do not include race. At this point in time, it is unclear if corrections by race should be made. Pottel and colleagues made a strong pitch against the inclusion of race using population-normalized serum creatinine to develop the full age spectrum formula (see Table 2) [204].

Sex-Related Correction in Kidney Function and Disparities in Disease Progression

Whereas it is debatable if adjustments have to be made for race, or whether the poor performance of some formulae in different parts of the world is more related to the lack of international collaboration to establish a world formula; sex has to be considered when measuring kidney function. There are substantial sex differences for almost all biological scenarios. Females have less muscle mass as demonstrated in autopsy studies [205]. There is also a difference in the fat mass, especially during adolescence [206]. In the growing adolescent, there are differences in CysC levels by sex, as noted in the National Health and Nutrition Examination Survey [207]. In this third USA-representative study, cross-sectional CysC was higher in males (mean 0.84 mg/l), as well as in non-Hispanic White versus non-Hispanic Black and Mexican American adolescents ages 12–19. Furthermore, serum creatinine (mean 0.71 mg/dl) was also higher in males and lower in non-Hispanic Whites and Mexican Americans compared to non-Hispanic Black Americans. Furthermore, while creatinine increased with age, CysC levels decreased, particularly in females. Uric acid and blood urea nitrogen were significantly associated with CysC levels (adjusting for age, sex, and race/ethnicity) [207]. In a longitudinal sample of children and adolescents who underwent amalgam dental work in Lisbon, Portugal, the sex differences were not noted [208].

The decline in eGFR is greater among females compared to males, however, this may be explained by the CKD etiology, as females have more glomerular conditions compared to males, who have more congenital anomalies of the kidney and urinary tract (CAKUT) [209]. The disparities by sex in patients aged 2–19, continue at end-stage kidney disease. In the United States Renal Data System, 14, 024 pediatric patients started renal replacement therapy between 1995 to 2011, and girls had a greater risk of death or were less likely to receive a transplant in an adjusted analysis [210].

We analyzed the difference by sex when developing a BTP formula, comparing the correlation of creatinine with this small molecular weight protein [142]. Figure 3 shows the regression lines for the reciprocal of CysC, in adolescents with CKD (165 males and 155 females) aged 13–18 years. Whereas the slopes were similar, the elevations of the two regression lines were significantly different ($F = 18.90$, DFn = 1 DFd = 317, $p < 0.0001$, *unpublished data*).

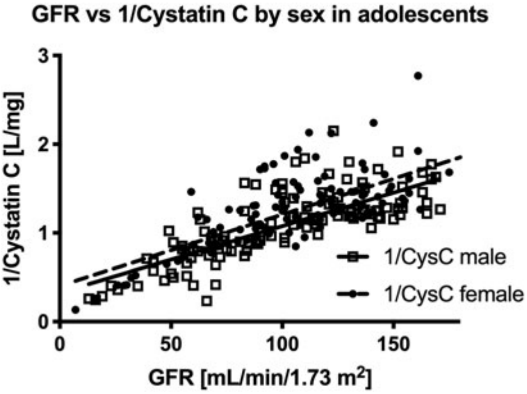

Fig. 3 The relationship between measured ^{99m}Tc DTPA GFR and the reciprocal of 1/Cystatin C in adolescents with chronic kidney disease (165 males and 155 females) age 13–18 years. Slopes were similar but the elevations of the two regression lines were significantly different, indicating the strong need for sex-specific formulae ($F = 18.90$, DFn = 1 DFd = 317, $p < 0.0001$)

Imaging for the Measurement of Kidney Function

Imaging has been proposed as an alternative for the assessment of kidney function. Magnetic resonance imaging (MRI) may serve as a good tool to assess interstitial fibrosis in kidney allografts [211]. Several authors, especially Abitbol from Miami, have advocated for using ultrasound-based renal volumes and renal volume z-scores for the assessment of kidney function [212]. This may work well for neonates in whom there is no hyperfiltration, however, there may be glomerular hypertrophy and an increase of the renal cortex, which masks decreased kidney function. Renal length z-score by ultrasound measurement in children with solitary functioning kidneys does not predict kidney function at 7 years of age (mean follow up of 5 years) [193]. In an elegant renal ultrasound study, it was shown that only the medulla, as a separate compartment, lags behind after premature birth [213]. Ultrasound images were obtained at 32 weeks, 37 weeks, and 6 months of age. Kidney volume, length, renal cortex and medulla thickness were measured and compared between preterm and term babies. Traditionally, it is even difficult to get renal volumes from radiologists. Kidney length or kidney volume z-scores are rarely reported, let alone reports of the differentiation of the volumes of the medulla and cortex. We have recently assessed the relationship between kidney length and kidney volume z-scores and found very poor agreement [215]. On the other hand, special CTs may evolve to become a tool for measuring both renal cortex and renal medulla blood flow, as we have recently shown when analyzing the impact of testosterone on renal function [212]. At this point in time, imaging is not yet well established as a tool for the measurement of kidney function; however, here is a great opportunity for future research.

Conclusions

Kidney function cannot be assessed directly and GFR is the best surrogate of renal function. The gold standard for GFR measurement is inulin clearance (ideally combined with simultaneous PAH clearance); however, this is no longer practiced in the clinical setting. Fortunately, there is good agreement with nuclear medicine methods such as ^{99}Tc iohexol ^{99}Tc DTPA, ^{51}Cr EDTA, and iothalamate. Iohexol and iothalamate can also be performed without radiolabeling.

Creatinine, albeit a marker afflicted with many technical problems, is the most commonly used endogenous biomarker of kidney function. CysC is a superior biomarker to measure kidney function and can be used even at 1 day of life, but its availability is limited. Currently, the best tool for estimation of GFR is the combination of CysC and creatinine measurements. Special populations require customized approaches for measurement and interpretation of kidney function. Future research may involve additional endogenous biomarkers, such as BTP, B2M, and TMAP. The role of imaging for the assessment of kidney function requires additional research.

Cross-References

- ▶ Drug Dosing in Abnormal Kidney Function in Children
- ▶ Evaluation and Management of Acute Kidney Injury in Children
- ▶ Nutrition, Growth, and Development
- ▶ Postnatal Renal Maturation
- ▶ Progression of Chronic Kidney Disease and Nephroprotection in Children

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Imaging Kidneys and the Urinary Tract

6

Jonathan Loewen and Larry A. Greenbaum

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Abstract

Diagnostic imaging is an essential component of the routine evaluation of children with kidney disease. Ultrasound, due to low cost and absence of side effects, is the most common modality, with special importance in evaluating a child with acute kidney injury or hematuria and diagnosing and following children with congenital anomalies of the kidney and urinary tract. Voiding cystourethrograms remain important for diagnosing vesicoureteral reflux and assessing the urethra, but radiation exposure can be reduced or eliminated in some patients via radionuclide cystography or contrast-enhanced voiding urosonography, respectively. CT, ideally with protocols that reduce radiation exposure, provides excellent anatomic resolution, with applications that include assessing children for renal artery stenosis and acute nephrolithiasis, and evaluating a child following trauma to the urinary tract. MRI provides excellent anatomical detail without radiation but is expensive and may require sedation in young children. Magnetic resonance urography enables determination of split differential renal function and transit time of contrast through the kidney, making it an effective technique for assessing hydronephrosis. A variety of nuclear medicine modalities are used to assess renal scarring, locate ectopic kidneys, decide if a hydronephrotic kidney is obstructed, locate a pheochromocytoma, and determine differential renal function. Retrograde urethrography, often utilized after trauma, evaluates urethral caliber and integrity.

Keywords

Radiograph · Fluoroscopy · Ultrasound · CT · MRI · Nuclear medicine

Synonyms

Hydronephrosis; Urinary tract dilation

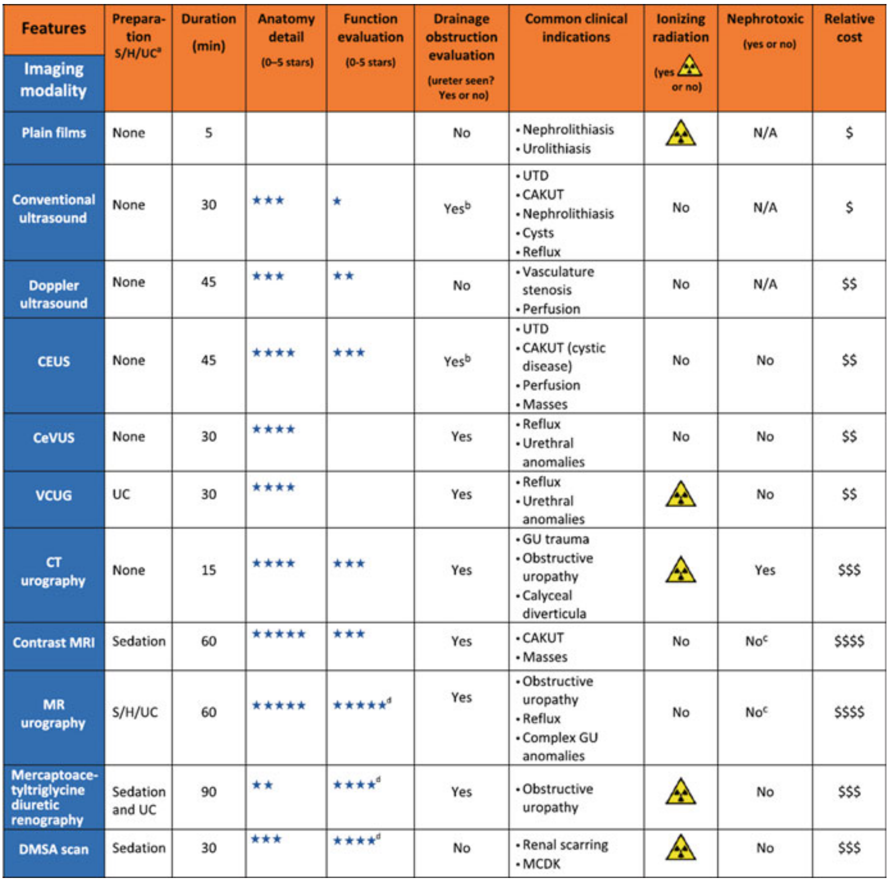
Diagnostic Procedures

Imaging is essential for the diagnosis and management of many disorders of the urinary tract (UT). Clinicians should be familiar with the available techniques, including their indications, limitations, and potential complications [1, 2]. Ultrasound (US) is the most widely used modality because of the information provided, safety, and low cost. Intravenous pyelography (IVP) is currently seldom utilized, but voiding cystourethrography (VCUG) and nuclear medicine studies continue to have important roles. Computed tomography (CT) is readily available and can acquire images rapidly, generally obviating the need for sedation. Magnetic resonance imaging (MRI) generates anatomically precise images without radiation exposure, but at high direct financial cost and with the need for sedation in young children. Imaging studies should be carefully selected, with consideration of diagnostic utility, cost, and potential complications such as contrast nephropathy and radiation exposure (Table 1).

Ultrasound

US is the principal imaging modality for visualization of the UT. US provides a rapid assessment of kidney shape and size, and it is especially good at identifying hydronephrosis. Studies are completed without discomfort or sedation, and there are no known complications or side effects. In addition, US is relatively inexpensive and readily

Table 1 Summary of exam duration, indication, relative cost, and presence/absence of ionizing radiation



Features Imaging modality	Preparation S/H/UC[a]	Duration (min)	Anatomy detail (0–5 stars)	Function evaluation (0-5 stars)	Drainage obstruction evaluation (ureter seen? Yes or no)	Common clinical indications	Ionizing radiation (yes ☢ or no)	Nephrotoxic (yes or no)	Relative cost
Plain films	None	5			No	• Nephrolithiasis • Urolithiasis	☢	N/A	$
Conventional ultrasound	None	30	★★★	★	Yes[b]	• UTD • CAKUT • Nephrolithiasis • Cysts • Reflux	No	N/A	$
Doppler ultrasound	None	45	★★★	★★	No	• Vasculature stenosis • Perfusion	No	N/A	$$
CEUS	None	45	★★★★	★★★	Yes[b]	• UTD • CAKUT (cystic disease) • Perfusion • Masses	No	No	$$
CeVUS	None	30	★★★★		Yes	• Reflux • Urethral anomalies	No	No	$$
VCUG	UC	30	★★★★		Yes	• Reflux • Urethral anomalies	☢	No	$$
CT urography	None	15	★★★★	★★★	Yes	• GU trauma • Obstructive uropathy • Calyceal diverticula	☢	Yes	$$$
Contrast MRI	Sedation	60	★★★★★	★★★	Yes	• CAKUT • Masses	No	No[c]	$$$$
MR urography	S/H/UC	60	★★★★★	★★★★★[d]	Yes	• Obstructive uropathy • Reflux • Complex GU anomalies	No	No[c]	$$$$
Mercaptoacetyltriglycine diuretic renography	Sedation and UC	90	★★	★★★★[d]	Yes	• Obstructive uropathy	☢	No	$$$
DMSA scan	Sedation	30	★★★	★★★★[d]	No	• Renal scarring • MCDK	☢	No	$$$

From Viteri et al. [3]

available. The skill of the examiner can significantly influence the information obtained via US. Lack of patient cooperation and large body habitus may also limit image quality. Because of its low cost and the absence of side effects, US is the optimal modality for clinical situations requiring repeated evaluations. For example, US can assess hydronephrosis before and after an intervention such as a catheter, stent, or nephrostomy tube placement. US is also ideal as a screening examination for children who need surveillance for kidney stones or malignancy. Advances in sonographic technology including compound imaging, harmonic imaging, panoramic imaging, elastography, 3D imaging, and contrast-enhanced sonography/voiding urosonography have improved image quality and utility [3].

Technique

Images are generally obtained with the patient supine, which optimizes visualization of the upper poles, using the spleen and liver as acoustic windows. The contralateral decubitus position may permit better visualization in some patients. In certain situations, placing the patient in the prone position may allow for better visualization of the lower pole if subtle pathology is present. Ideally, the bladder should be visualized while filled with urine. A cooperative child should be asked not to void for a few hours prior to the exam. In a patient who is not toilet trained, the bladder should be visualized first to allow imaging of the bladder before the patient voids. US transducers produce sound waves of varying frequencies. High-frequency sound waves do not penetrate tissue as deeply but provide greater resolution. In most situations, the sonographer should use the highest frequency that penetrates to the desired depth. Hence, high-frequency US permits excellent resolution in infants and small children. Conversely, resolution is decreased in very large or obese individuals.

US evaluation of the UT should include measurement of renal lengths; assessment of renal shape, thickness and echogenicity of the cortex, and the presence of corticomedullary differentiation; and evaluation of the renal pelvis, ureters, and bladder. The ureters are not usually visible but may be seen when dilated due to underlying pathology. There are published standards for US determination of renal length for fetuses, preterm infants, full-term neonates, infants, and older children (Table 2) [4–9]. Length measurements should be made with the patient supine or in the contralateral decubitus position; length may be underestimated with the patient prone [10, 11]. Gestational age and birth weight correlate with kidney length in newborns [4, 7]. In older children, age is a convenient parameter, but length or height is a better predictor of normal kidney length [6, 12, 13]. A multivariable approach allows for improved prediction equations for renal length, including a web-based calculator [14]. In addition to renal length, normative values have been established for pediatric renal volumes and urinary bladder volumes (Figs. 1 and 2) [15].

Table 2 Renal length

Age	Mean length (cm)	Range (+/− 2 SD in cm)
Term newborn	4.48	3.86–5.10
2 months	5.28	3.96–6.60
6 months	6.15	4.81–7.49
1.5 years	6.65	5.57–7.73
2.5 years	7.36	6.28–8.44
3.5 years	7.36	6.18–8.54
4.5 years	7.87	6.87–8.87
5.5 years	8.09	7.01–9.17
6.5 years	7.83	6.39–9.27
7.5 years	8.33	7.31–9.35
8.5 years	8.90	7.14–10.66
9.5 years	9.20	7.40–11.00
10.5 years	9.17	7.53–10.81
11.5 years	9.60	8.32–10.88
12.5 years	10.42	8.68–12.16
13.5 years	9.79	8.29–11.29
14.5 years	10.05	8.81–11.29
15.5 years	10.93	9.41–12.45
16.5 years	10.04	8.32–11.76
17.5 years	10.53	9.95–11.11
18.5 years	10.81	8.55–13.07

Adapted from Rosenbaum et al. [9]

In adults and children, the average left kidney is slightly longer than the average right kidney [6, 7, 12], although this is only observed in slightly more than 50% of measurements of individual patients (the remainder have kidneys of equal length or a longer right kidney) [13]. In infants and adults, the median left renal length is about 3 mm longer than the median right renal length [6, 16]. In adults and older teenagers, males have longer kidneys, correlating with their increased height compared to females. Black children have shorter kidneys than white children [17]. Measurement of renal length in children has a fairly high rate of intraobserver and interobserver variation [18]; hence, US measurement of renal length lacks the accuracy to allow assessment of renal growth over limited time periods. Measurement of the left kidney length is less accurate, probably because the spleen provides a smaller acoustic window than the liver. Nevertheless, renal length discrepancy in children can be used to predict abnormalities on technetium 99m-dimercaptosuccinic acid (^{99m}Tc-DMSA) renal scintigraphy [19].

The appearance of the kidneys changes with age. The relative volume of medullary tissue compared to cortical tissue is increased in neonates and infants (Fig. 3a) [20]. Cortical echogenicity is increased in the term newborn kidney, with the majority having the same echogenicity as the liver or spleen [8]. Renal cortical echogenicity is further increased in premature infants, with some kidneys having cortical echogenicity greater than the echogenicity of the liver or spleen [21]. Cortical echogenicity decreases after birth, and only a small percentage of normal kidneys of term infants have cortical echogenicity equivalent to the liver or spleen by 6 months. Cortical echogenicity is compared to the liver or spleen, but this comparison may not be valid if there is underlying liver disease. Transient

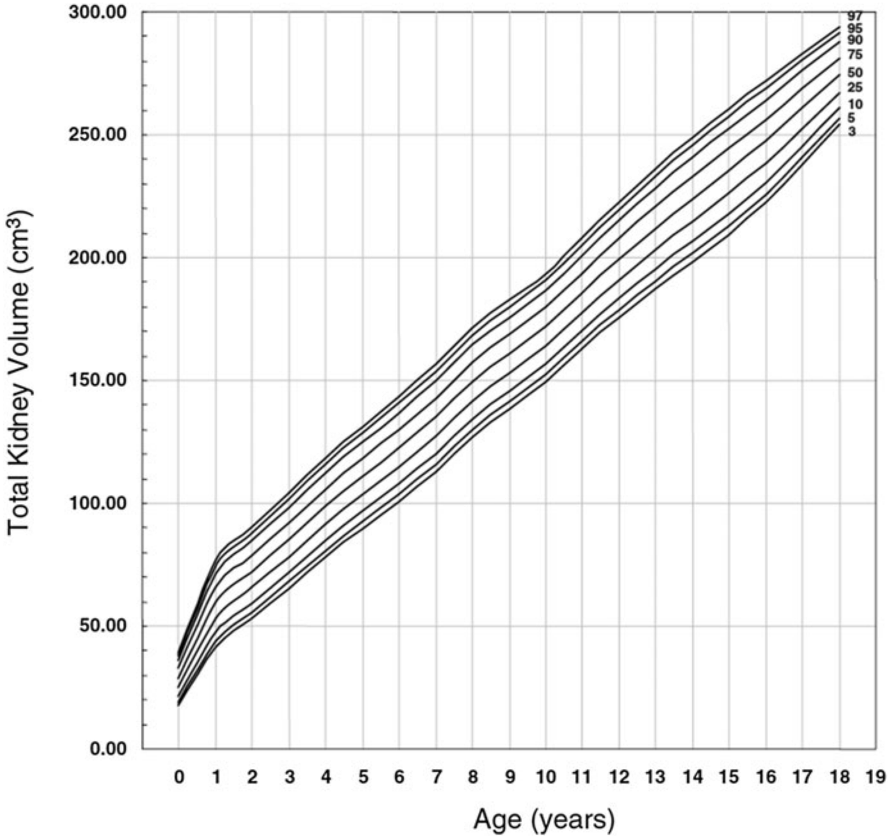


Fig. 1 Smoothed centiles of total kidney volume (length × width × depth × 0.523) in 3,376 children. (From Leung et al. [15])

increased cortical echogenicity of the kidneys may occur in children with a variety of nonrenal illnesses [22]. The kidneys of term newborns have prominent medullary pyramids that are relatively hypoechoic [20]. This results in marked corticomedullary differentiation. The echogenicity of the medullary pyramids increases after birth, and prominent medullary pyramids are not normally present by 1 year. In contrast, the central sinus echo is minimal at birth and gradually increases during the first 10 years of childhood (Fig. 3b, c) [8].

Hydronephrosis may indicate obstruction or vesicoureteral reflux (VUR), or it may be a normal variant, especially when mild. Several classification systems for hydronephrosis exist, including the Society for Fetal Urology grading system, as well as the Urinary Tract Dilation (UTD) grading system for hydronephrosis [23]. The Society for Fetal Urology grading system for hydronephrosis is predictive of the presence or absence of obstruction (Table 3) [24, 25]. Mild hydronephrosis is common in newborns and occurs in a higher percentage of males than females [26]. The UTD grading system was devised to provide more uniformity in nomenclature, have a more consistent means of classifying prenatal/postnatal urinary tract dilation, and to provide an algorithmic approach to postnatal management [23].

An increase in bladder wall thickness may be an indication of pathology, especially chronic increased bladder pressure. Measurement of bladder wall thickness is highly dependent on the degree of bladder filling, with some variation based on location of measurement, body mass index, and age [27]. The upper limit of normal is 5 mm for an empty bladder and 3 mm for a well-distended bladder [28]. US can be used to accurately estimate bladder volume [29, 30]. US

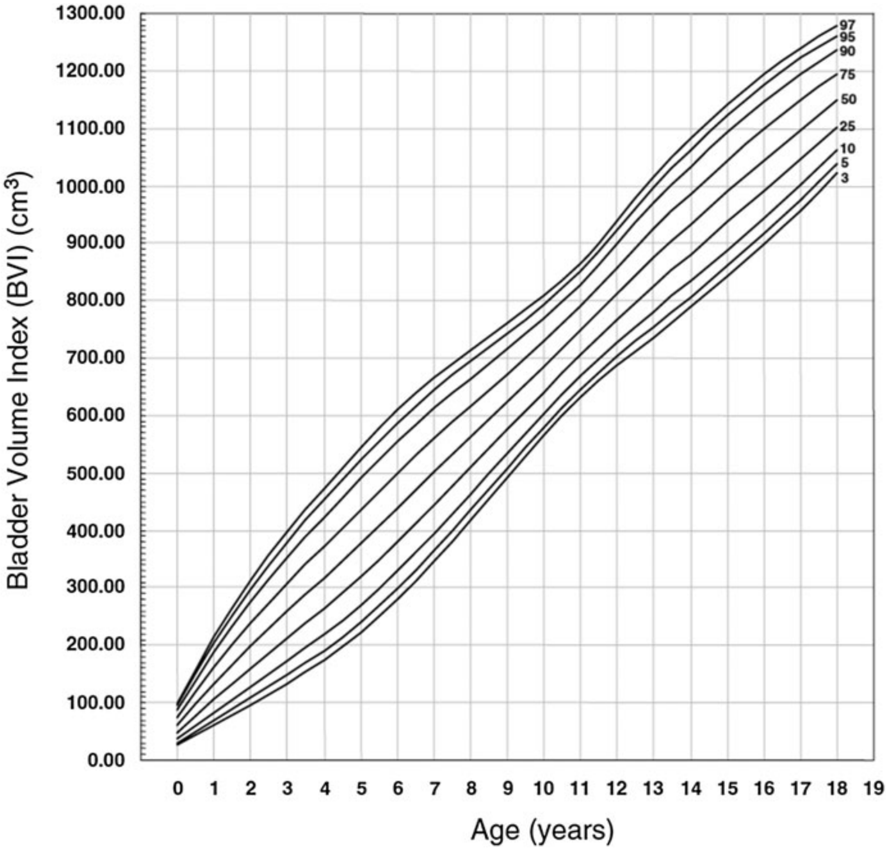


Fig. 2 Smoothed centiles of bladder volume index (length × width × depth) in 3.376 children. (From Leung et al. [15])

measurement of bladder volume after voiding is a useful parameter for assessing bladder function. US may detect ureteroceles or bladder masses.

Doppler US permits evaluation of blood flow and has been utilized to identify renal artery disease, renal vein thrombosis, tumor thrombosis in the renal vein and inferior vena cava, and arteriovenous fistulas [31, 32]. Resistance to blood flow can be quantified by measuring the resistive index (RI), which is calculated from the formula: RI = (peak systolic velocity-end diastolic velocity)/peak systolic velocity. A normal RI is below 0.7 in adults and children who are 6 years of age or older, but higher values are observed in children less than 6 years [33]. Power Doppler mode allows increased sensitivity for blood flow detection but does not provide information on flow direction or velocity. Power Doppler US is superior to color Doppler US in detecting intrarenal blood flow [34] and in identifying areas of decreased perfusion within the kidney [35]. Power Doppler increases the sensitivity of US for detecting acute pyelonephritis [36]. Power Doppler is very sensitive to motion artifact; hence, the requirement for a very cooperative patient limits its utility in young children. 3D US of the kidneys can also be performed, with subtraction of a dilated collected system in order to calculate renal parenchymal volumes [37].

Alternative sonographic techniques include contrast-enhanced US (CEUS) and contrast-enhanced voiding urosonography (CEVUS), each of which requires scanners that have a contrast imaging package/mode and administration of an US contrast agent consisting of microspheres containing an inert gas [38]. CEUS requires the contrast agent to be administered intravenously and can be used to assess perfusion of both native

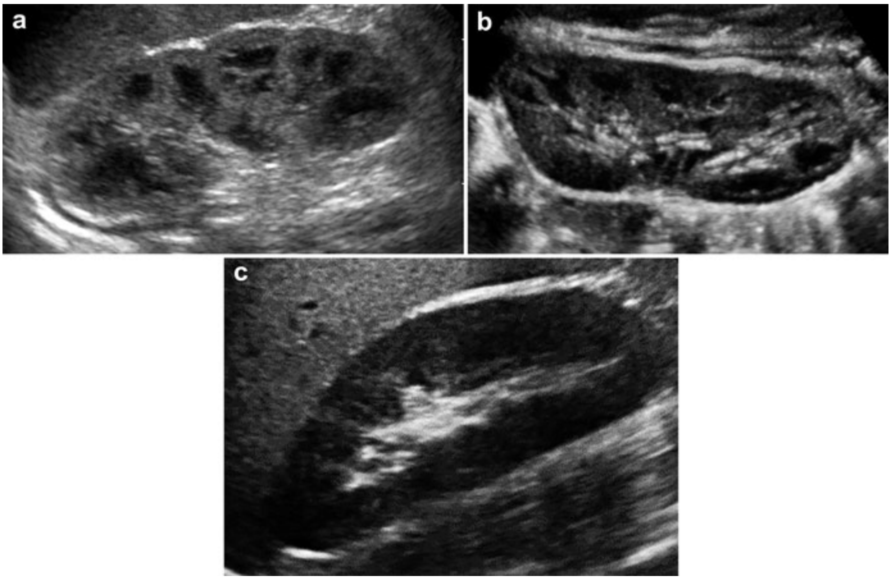


Fig. 3 (**a**) Normal ultrasound of a neonatal kidney. Medullary pyramids are hypoechoic with a relatively thin cortex. (**b**) Normal 2-year-old kidney. (**c**) Normal 16-year-old kidney. Note decreasing cortical echogenicity, decreased conspicuity of medullary pyramids, and increased renal sinus fat echoes with increasing age

Table 3 Society of Fetal Urology grading system for hydronephrosis

Grade	Ultrasound
0	No hydronephrosis
1	Only renal pelvis is seen
2	Renal pelvis and a few calices are seen
3	Virtually all calices are seen
4	Similar to grade 3, but parenchymal thinning present

and transplanted kidneys [3]. CEVUS is an alternative technique for detecting VUR that uses intravesical instillation of the US contrast agent [39–41]. The principal advantage is the elimination of radiation exposure, but bladder catheterization is still necessary. The results with VUS correlate well with conventional VCUG [40, 42, 43] and the technique can be used to image the urethra [3, 41].

Application

US is the initial imaging modality in a variety of clinical situations, including evaluation of renal failure, kidney transplant dysfunction, hematuria, screening for renal malignancy, abdominal mass, and follow-up of abnormal prenatal US. Procedural applications of US in pediatric nephrology include pre-biopsy or real-time localization of native and transplant kidneys and venous localization prior to hemodialysis catheter placement [44].

Voiding Cystourethrography

VCUG is the gold standard for the diagnosis of VUR. Most clinical studies of VUR have utilized VCUG. Moreover, VCUG is the only modality that detects VUR and provides detailed information about the bladder and urethra. Radiation exposure is the most important side effect of VCUG. Over the last several years, the radiation dose has been reduced through technological innovations, and a variety of additional approaches are available to further reduce radiation exposure [45–47]. A known allergy to contrast material is a contraindication to the study [48]. Because of the need for catheterization, VCUG creates a small risk of iatrogenic infection and trauma to the urethra, although these risks are low [49]. In addition, many children and families find catheterization frightening and emotionally traumatic. Sedation with midazolam appears safe and effective [50].

Technique

VCUG is done using fluoroscopy. An initial film prior to bladder catheterization detects calcifications or bony abnormalities; an abnormal bowel gas pattern may suggest a mass. The bladder is sterilely catheterized using a feeding tube (5 F in young infants; 8 F in older children), and urine can be obtained for culture. After emptying the bladder of urine, contrast material is infused to fill the bladder based on estimates of bladder capacity, but using gravity to avoid overfilling. The bladder must be filled to capacity to insure an adequate study. An early film during filling of the bladder is useful for identifying filling defects, such as caused by a ureterocele or bladder tumor. Films are taken before and after voiding, with VUR graded based on the International Reflux Study in Children [51]. In young children, who may not allow their bladder to fill to capacity, the sensitivity of VCUG for detecting VUR improves with multiple cycles of bladder filling and voiding [52, 53], but this increases the length and radiation exposure of the procedure. A film during voiding permits visualization of the urethra and is essential in male children to assess for posterior urethral valves (Figs. 4 and 5). At the end of voiding, a final film identifies residual urine and previously unidentified VUR. Technological advances including digital fluoroscopy with pulsed fluoroscopy and last-image-hold options have made it possible to diminish radiation dosage [54].

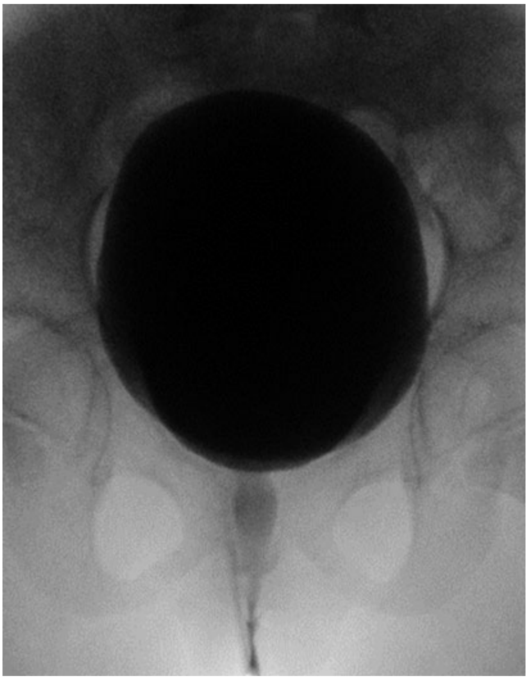


Fig. 4 Voiding cystourethrogram. Frontal voiding image of normal 5-year-old girl

Intravenous Pyelography

The availability of US and other imaging modalities such as MR urography has dramatically decreased the use of intravenous pyelography (IVP). The risks of IVP include radiation and contrast exposure; intravenous (IV) access is necessary.

Technique

An initial supine film is performed prior to contrast injection. Approximately 5–7 min after contrast, a film centered on the kidneys allows visualization of the nephrogram and the calices. Subsequent films are full length and taken 15 min and 30 min after injection. Delayed films are useful in cases of urinary obstruction. Bowel preparation does not improve image quality, although a short fasting period is usually recommended. The radiation exposure of IVP can be reduced by adjusting the number of films based on the clinical question and the results of initial films. Nonionic contrast medium improves image quality and decreases the risk of contrast nephropathy.

Application

Poor renal function limits the utility of IVP due to inadequate contrast accumulation in the kidneys. Moreover, poor renal function increases the risk of contrast nephropathy. IVP is currently rarely used in the evaluation of obstructive disease; US and nuclear medicine studies are the preferred imaging modalities. ^{99m}Tc-DMSA has replaced IVP for detection of renal scarring. IVP is useful for evaluation of ectopic ureters, ureteroceles, and fusion anomalies, although alternative imaging (US, MRI, CT) is generally utilized when available.

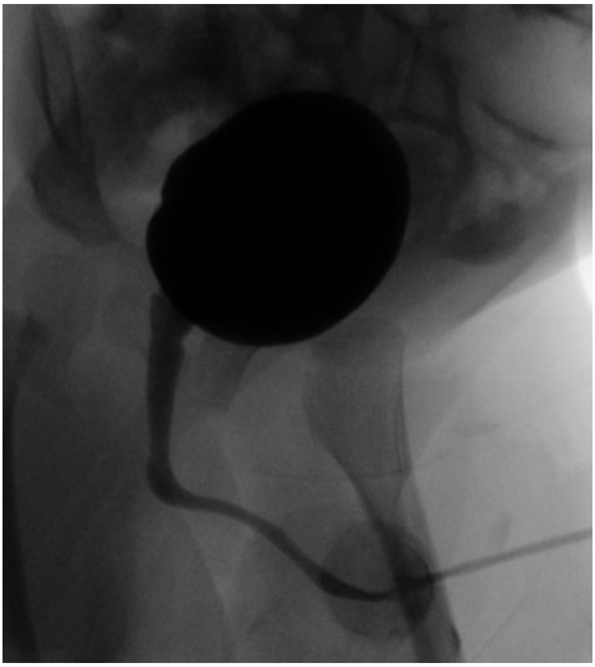


Fig. 5 Voiding cystourethrogram. Oblique voiding image of a normal 1-year-old boy

Retrograde Urethrography

Retrograde urethrography (RUG) is performed to evaluate urethral caliber and integrity, frequently following trauma [55].

Technique

Following advancement of a balloon microcatheter through the urethral meatus, the balloon is insufflated and iodinated contrast material is injected. The exam is typically performed fluoroscopically, but can be performed using convention radiography when needed [55].

Application

RUG is most frequently performed following perineal/pelvic injury, when there is concern for traumatic urethral injury, characterized by contrast extravasation (Fig. 6). The technique can also be used to evaluate for urethral stricture [55].

Computed Tomography

CT provides excellent anatomic resolution of the UT (Fig. 7) and has many applications. Radiation exposure is an important risk of CT and should limit its use to cases where the detail provided outweighs the risk of the study. IV contrast is necessary for optimal studies in most situations. Multiple strategies and innovations have reduced the radiation dose with CT [56, 57], but it remains an important concern. Sedation is currently rarely necessary due to rapid image acquisition, which also decreases motion artifact [58].

Technique

Thin-section helical imaging, now performed on all CT scanners, allows for multiplanar reformatted images to be obtained quickly and displays the kidneys, ureters, and bladder in three planes. Oral contrast, while often unnecessary for evaluation of the UT, is useful for examinations where differentiating bowel from pathology is necessary. Time constraints (acute trauma) or risk of aspiration may prevent the use of oral contrast. For younger children, excessive movement can be prevented by using blankets or adhesive tape. IV contrast is usually indicated, with the notable exception of nephrolithiasis evaluation. Low osmolality nonionic contrast is preferred; contrast is dosed based on weight. Neonates and infants require a longer delay from contrast injection to imaging.

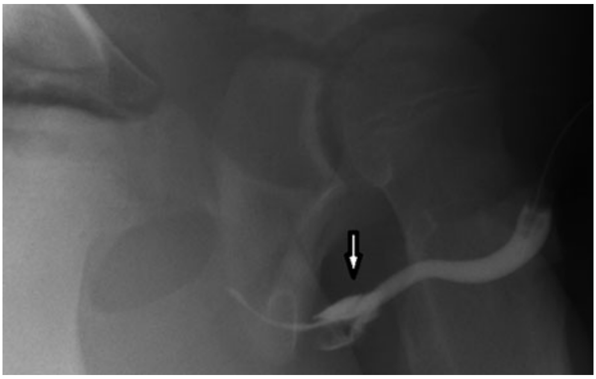


Fig. 6 Retrograde urethrogram of 10-year-old male following handlebar injury to perineum. Note urethral extravasation (arrow)

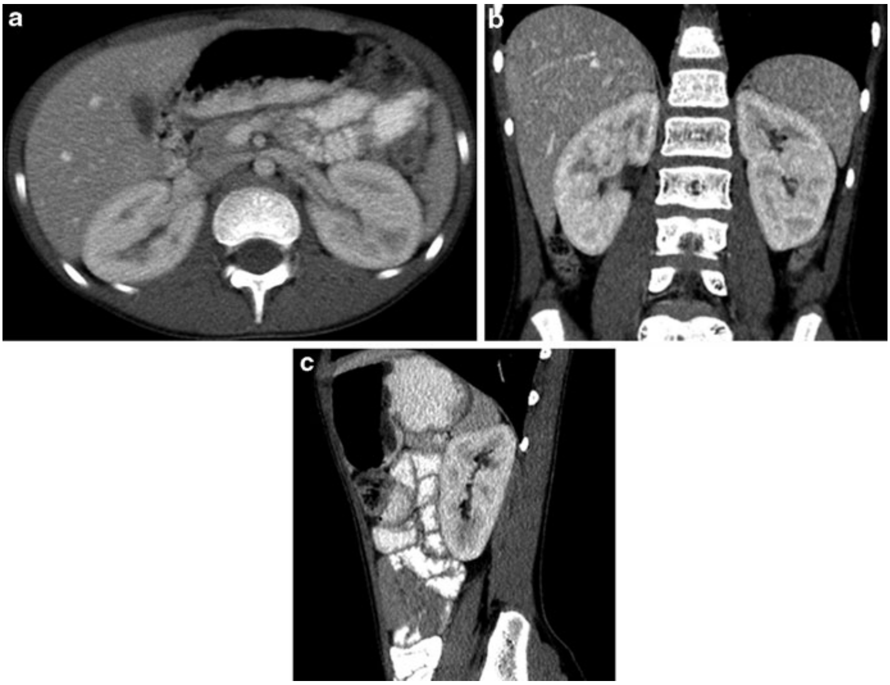


Fig. 7 CT of a normal 11-year-old boy. (**a**) Axial image through the midpole of both kidneys. (**b**) Coronal image. (**c**) Sagittal image

Application

CT is the most sensitive modality for initial evaluation of possible symptomatic nephrolithiasis, although US is generally utilized for surveillance to minimize radiation exposure. CT is used for renal tumor staging and is the optimal modality when trauma of the UT is suspected. CT provides excellent resolution of renal scarring, but ^{99m}Tc-DMSA is more commonly used. CT angiography is useful in assessing for renal artery stenosis [59]. Diminishing radiation exposure by using age- or weight-based exposure parameters and by limiting the field of view is suggested. Efforts should be made to achieve the lowest radiation dose needed in order to generate a diagnostic exam [56, 57].

Nuclear Medicine

A variety of radiotracers are utilized for imaging of the UT [2, 3]. The radiotracers emit photons, which are detected by a scintillation detector. Nuclear medicine imaging does not provide anatomic detail, but rather functional data that is typically utilized to answer a specific question. Nuclear medicine is often a complementary technique to imaging that provides structural information. While there is radiation exposure, it is less than other modalities such as VCUG, IVP, or CT. Glomerular filtration rate can be measured following the injection of a radiopharmaceutical and is discussed in detail in Chapter 21 [60].

Technique

Dynamic Renal Scintigraphy

Technetium 99m-diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) and technetium 99m-mercaptoacetyltriglycine (^{99m}Tc-MAG$_3$) are the radiotracers currently utilized for dynamic renal scintigraphy. ^{99m}Tc-DTPA is primarily excreted via glomerular filtration; it is not reabsorbed or secreted by the tubules; its clearance is slightly lower than inulin. ^{99m}Tc-MAG$_3$ is predominantly excreted via tubular secretion. For most indications, ^{99m}Tc-MAG$_3$ is superior to ^{99m}Tc-DTPA because ^{99m}Tc-MAG$_3$ is more rapidly excreted and thus provides a better renal/background ratio. The nuclear radiotracers have a lower allergic reaction profile than radiographic contrast media [61].

Patients are encouraged to consume liquids immediately prior to the procedure as this facilitates exam interpretation and decreases the radiation dose to the urinary bladder [62]. Patients should ideally void before injection of isotope. The uncooperative child should be immobilized, but sedation is rarely necessary. Images are obtained with the patient supine; the scintillation detector is below the patient for native kidneys and above the patient for renal allografts. After bolus injection of radiotracer, dynamic images are recorded for 30 min. The initial images assess renal perfusion and later images evaluate excretion. Differential renal function is calculated using the early images, typically during the second minute, prior to radiotracer appearance in the collecting system.

Diuresis scintigraphy is performed to evaluate a patient for renal obstruction [63, 64]; use of a diuretic is only necessary if radiotracer does not rapidly drain from the renal pelvis during initial imaging. A dilated renal pelvis that is not obstructed may have slow drainage of radiotracer due to the increased pelvic volume. Administration of diuretic allows differentiation of an obstructed kidney from a non-obstructed dilated renal pelvis. Furosemide is given 15–20 min after administration of radiotracer, after which radiotracer drains rapidly from a non-obstructed renal pelvis (Figs. 8 and 9). Conversely washout of radiotracer is limited if obstruction is present; a half-life of radiotracer disappearance greater than 20 min indicates obstruction [65]. Test results and histological changes correlate well [66], and the test is sensitive and specific [65, 67]. Diuresis

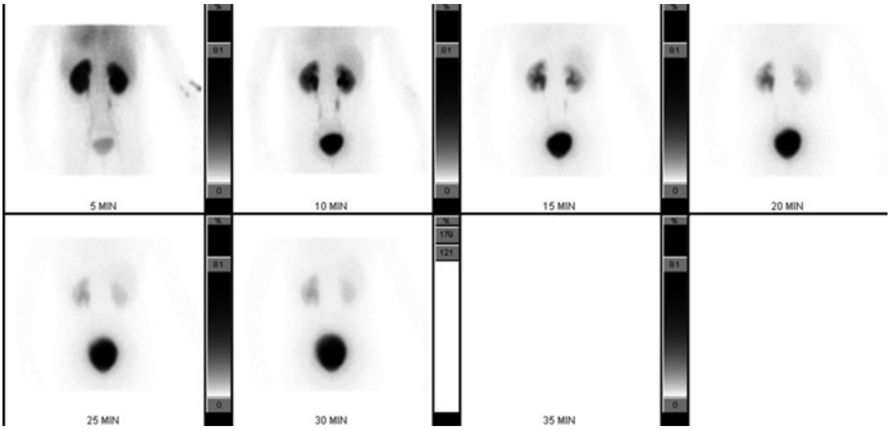


Fig. 8 Posterior image of a normal ^{99m}Tc-MAG$_3$ renal scan in an 8-year-old with hydronephrosis by ultrasound

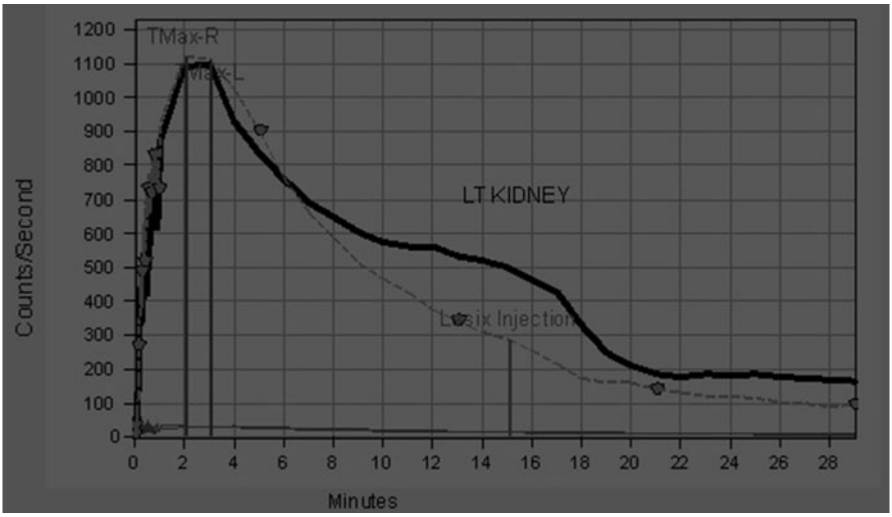


Fig. 9 Time-activity curve from ^{99m}Tc-MAG$_3$ renal scan showing symmetric prompt excretion of radiotracer, indicating no obstruction

scintigraphy is accurate in newborns, despite their lower glomerular filtration rate [67, 68], although follow-up imaging of normal results is necessary to ensure that obstruction does not subsequently develop [69]. Potential causes of false-positive results are a massively dilated renal pelvis or a kidney with very poor function [70], although technical modifications may allow for more accurate assessment of a poorly functioning kidney [71, 72]. False-positive results can also be decreased by obtaining images after the patient voids to avoid bladder pressure effects and after placing the child in the erect position to maximize gravitational drainage [73, 74].

Cortical Scintigraphy

^{99m}Tc-DMSA is the preferred radiotracer for imaging of the renal cortex [75]. ^{99m}Tc-DMSA is taken up by the cells of the proximal tubule, where it binds to sulfhydryl groups (Fig. 10). The accumulation of ^{99m}Tc-DMSA in the kidney is relatively slow; hence, imaging is typically performed 1.5–3 h after injection, although diagnostic accuracy may improve with an increased delay in imaging if renal function is significantly impaired. Posterior and left and right posterior oblique views should be obtained, although loss in diagnostic accuracy is minimal if only a posterior view is possible in a restless child [76]. Anterior imaging is preferred for pelvic or horseshoe kidneys. Additional pinhole images are useful for improving resolution when imaging infants.

A variety of factors may complicate interpretation of ^{99m}Tc-DMSA imaging. Fetal lobulations may give the false impression that there are areas of decreased radiotracer uptake. The splenic impression explains flattening of the superolateral surface of the left kidney [77]. The poles of normal kidneys may have relatively decreased uptake of radiotracer. Interobserver agreement in the interpretation of ^{99m}Tc-DMSA scans for scarring and acute pyelonephritis varies between studies [78–80]. ^{99m}Tc-DMSA is not specific for scars or acute pyelonephritis. Alternative explanations for defects such as cysts, hydronephrosis, infarcts, masses, or a dysplastic half of a duplex kidney can only be identified using alternative imaging strategies (e.g., US). Children with proximal tubulopathies have decreased uptake of ^{99m}Tc-DMSA and increased urinary excretion [81].

Single-photon emission computed tomography (SPECT) is an alternative to planar scintigraphy. SPECT appears to offer superior sensitivity for detecting renal scars [82, 83], although with a higher rate of false-positive results [84, 85]. The clinical relevance of the increased detection of small scars via SPECT is not clear. An intrarenicular septum, a common normal variant,

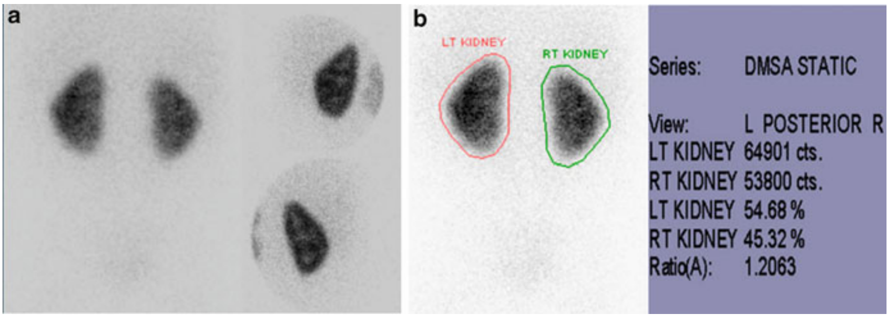


Fig. 10 (**a**) Posterior image and coned oblique images of normal kidneys on DSMA scan in a 3-year-old with a urinary tract infection. (**b**) Regions of interest drawn for split-function calculation

may lead to a photopenic area with SPECT, but can be differentiated from a scar because of preserved cortical uptake [86]. The increased imaging time required with SPECT may necessitate sedation in young children.

Split function is determined by outlining the appropriate regions of the kidneys and subtracting the activity of an appropriate background area in the posterior image. Normal split function is 50 % +/– 6 %. Estimates of split function must be corrected for attenuation if there is an anteriorly placed ectopic kidney. Such correction is not accurate with a pelvic kidney due to the effect of the pelvic bone. Imaging may need to be delayed for up to 24 h in an obstructed kidney because isotope accumulation in the renal pelvis may cause an error in estimating split function, with an overestimation of the function of the obstructed kidney if the image is obtained prematurely. A similar approach may be necessary for a non-obstructed, severely dilated renal pelvis, although injection of furosemide, with the subsequent diuresis clearing radiotracer from the dilated pelvis, is an alternative strategy. Estimates of split function are reliable, with good interobserver agreement, except in cases where renal function is significantly impaired [87].

Adrenal Gland Scintigraphy

Metaiodobenzylguanidine (MIBG), when labeled with either ^{131}I or ^{123}I, is used for the detection of catecholamine-secreting tumors, including pheochromocytomas and neuroblastomas [88]. MIBG, a guanethidine analog that structurally resembles norepinephrine, is taken up by sympathetic nerve cells. The patient should receive oral nonradioactive iodine before and after administration of radiolabeled MIBG to compete with free radioactive iodine, minimizing radioactive exposure to the thyroid gland. A variety of medications interfere with MIBG uptake into tissue and should be discontinued prior to MIBG imaging (Table 4). MIBG radiotracer is injected and images are obtained at various times depending on the isotope. MIBG accumulates in a variety of organs, including the liver, heart and salivary glands; isotope is detected in the kidney and bladder due to renal excretion. Tumors can be identified because of the stronger radiotracer uptake, which is typically focal. ^{123}I has a much lower radiation burden than ^{131}I and results in superior images [89].

Table 4 Drugs that interfere with uptake of MIBG

Propranolol
Labetalol
Calcium channel blockers
Phenylephrine
Pseudoephedrine
Tricyclic antidepressants
Phenothiazines
Monoamine oxidase inhibitors

MIBG Metaiodobenzylguanidine

Radionuclide Cystography

Radionuclide cystography (RNC) is an alternative to VCUG for the detection of VUR (Fig. 11) [3, 90]. In RNC, a catheter is placed into the bladder, and a solution containing a radioisotope is infused, with complete filling of the bladder. Since it allows for continuous monitoring, RNC is

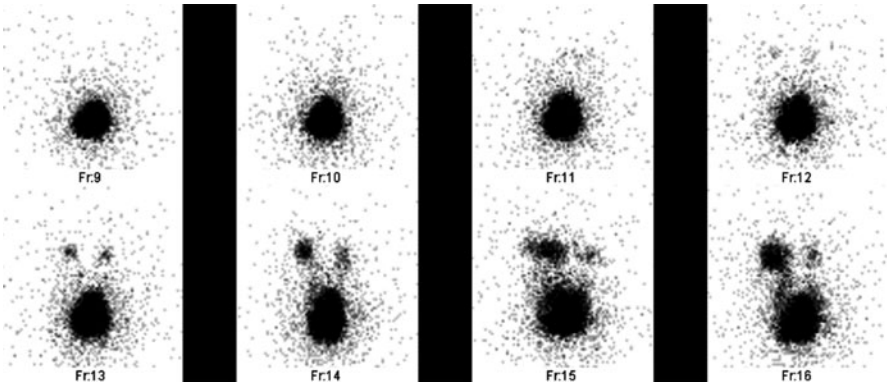


Fig. 11 Radionuclide cystogram. Posterior serial 10 s/frame images from a nuclear cystogram in a 4-year-old demonstrating bilateral vesicoureteral reflux

more sensitive at detecting VUR than VCUG, which cannot utilize continuous monitoring due to radiation concerns. Furthermore, the radiation burden with RNC is considerably less than VCUG. However, radionuclide cystography does not permit evaluation of the bladder or the urethra.

Applications

^{99m}Tc-DMSA is the preferred radiopharmaceutical for evaluation of the renal cortex and the gold standard for the identification of pyelonephritis and renal scars [62]. ^{99m}Tc-DMSA can be utilized to determine split renal function and to identify and characterize renal infarcts or anatomically abnormal kidneys such as horseshoe kidneys, pelvic kidneys, and crossed fused ectopia [91, 92]. Dynamic renal imaging with ^{99m}Tc-MAG$_3$ or ^{99m}Tc-DTPA permits evaluation of renal blood flow, split renal function, kidney location, and urinary drainage. Dynamic renal imaging allows determination of whether hydronephrosis is secondary to renal obstruction or an alternative etiology such as VUR, high urine flow, or relieved obstruction [93]. Dynamic renal imaging can be utilized to determine split renal function.

Magnetic Resonance Imaging

MRI, with its ability to image the abdomen in multiple planes and with sequences showing different signal characteristics, provides excellent resolution of the UT without radiation exposure (Figs. 12 and 13) [94]. Faster MR sequences allowing for rapid image acquisition, and the continued trend in reducing radiation exposure in the pediatric population have also increased imaging of the urinary tract by MRI [54]. Gadolinium-based contrast at standard doses is significantly less nephrotoxic than iodinated contrast [95], but gadolinium-based contrast is nephrotoxic when used at radiographic doses for angiographic procedures [96, 97]. Gadolinium-based contrast is also the dominant risk factor for the development of nephrogenic systemic fibrosis (NSF) [98], an entity that occurs almost exclusively in patients with end-stage kidney disease or severe renal impairment. Most cases of NSF have been associated with gadodiamide, although other gadolinium-based contrast agents may lead to NSF [99]. Gadolinium-based contrast should be used cautiously in patients with moderate to severe renal impairment [100]. Sedation is typically necessary for infants and young children. MRI is the most expensive imaging modality, but it may replace multiple other studies and provide superior resolution in certain clinical situations.

Technique

Magnetic resonance urography (MRU) provides superb anatomic detail combined with functional information [101]. In this technique, patients receive IV hydration and an IV gadolinium chelate (Gd-DTPA). The kidneys are imaged over time, allowing for a dynamic evaluation of renal

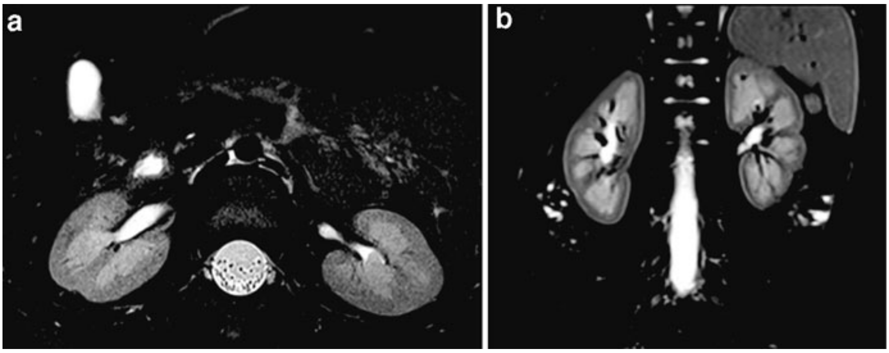


Fig. 12 Renal MRI of a normal 5-year-old. (**a**) Axial T2-weighted image through the midpole of the kidneys. (**b**) Coronal T2-weighted image

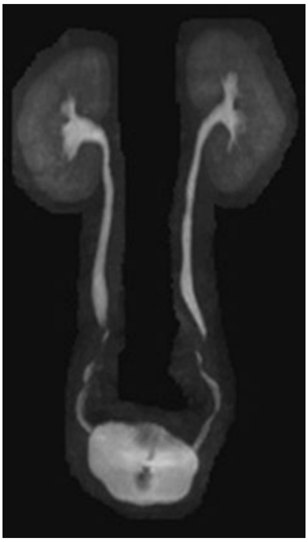


Fig. 13 Renal MRI. 3-D image of the kidneys and ureters in a normal 12-year-old

enhancement and drainage. In addition to anatomic assessment of renal parenchyma and collecting system anatomy, post-processing of MRU data derived from the study allows for determination of split differential renal function, transit time of the contrast agent through the kidney, and an estimate of glomerular filtration rate. MRU has superior contrast and temporal and spatial resolution when compared to renal scintigraphy [3, 54].

Application

Conventional MRI is useful in characterization of solid and cystic renal masses, renal vascular disease, and congenital abnormalities, including lower urogenital tract malformations and complex post-traumatic conditions [102–104]. The most common indication for MRU is hydronephrosis. This technique allows for simultaneous anatomic and physiologic assessment of the parenchyma, collecting system, and surrounding tissues [54, 105]. The goal of the exam is to determine whether the hydronephrotic kidney is obstructed based on its response to a fluid/diuretic challenge [101, 106].

Congenital Kidney and Urological Disorders

Fetal Imaging

The widespread use of fetal US has led to a dramatic increase in postnatal imaging of the UT. Most congenital abnormalities of the UT are now detected prenatally; this has led to a decrease in the diagnosis of obstructive uropathies as a consequence of urinary tract infection (UTI), potentially diminishing the need for renal US in children with UTI [107]. Fetal kidney length relates to gestational age (see Table 5) [5]. Fetal US is excellent at identifying hydronephrosis, which may identify infants with UT obstruction or VUR. Oligohydramnios, which may indicate poor urine output due to renal insufficiency, is easily quantified and followed. Persistent oligohydramnios is associated with pulmonary hypoplasia. The fetal bladder, which may be distended with impaired bladder emptying, and

Table 5 Mean renal lengths for various gestational ages

Gestational age (weeks)	Mean length (cm)	SD	95% CI	n
18	2.2	0.3	1.6–2.8	14
19	2.3	0.4	1.5–3.1	23
20	2.6	0.4	1.8–3.4	22
21	2.7	0.3	2.1–3.2	20
22	2.7	0.3	2.0–3.4	18
23	3.0	0.4	2.2–3.7	13
24	3.1	0.6	1.9–4.4	13
25	3.3	0.4	2.5–4.2	9
26	3.4	0.4	2.4–4.4	9
27	3.5	0.4	2.7–4.4	15
28	3.4	0.4	2.6–4.2	19
29	3.6	0.7	2.3–4.8	12
30	3.8	0.4	2.9–4.6	24
31	3.7	0.5	2.8–4.6	23
32	4.1	0.5	3.1–5.1	23
33	4.0	0.3	3.3–4.7	28
34	4.2	0.4	3.3–5.0	36
35	4.2	0.5	3.2–5.2	17
36	4.2	0.4	3.3–5.0	36
37	4.2	0.4	3.3–5.1	40
38	4.4	0.6	3.2–5.6	32
39	4.2	0.3	3.5–4.8	17
40	4.3	0.5	3.2–5.3	10
41	4.5	0.3	3.9–5.1	4

Gestational age is an average of the gestational ages in weeks determined on the basis of biparietal diameter, femoral length, and abdominal circumference. *cm* centimeters, *SD* standard deviation, *CI* confidence interval; *n* number of fetuses. From Cohen HL et al. [5]

the echogenicity of the renal parenchyma are also visualized via fetal US.

Hydronephrosis is the most common renal abnormality on fetal US, and ureteropelvic junction (UPJ) obstruction is the most common pathologic condition, although a high percentage of kidneys are ultimately diagnosed as normal [108]. A postnatal US done in the first few days of life to follow-up on prenatal hydronephrosis often yields a false negative result, presumably because of low urine output due to physiologic dehydration [109]. Hence, the initial postnatal US should be done at least 4 weeks after birth or the child should have a second US if an early postnatal US is negative. Deferral of the initial US is inappropriate if the prenatal US suggests the presence of disease that would require immediate intervention (e.g., bilateral hydronephrosis or oligohydramnios). The degree of prenatal hydronephrosis is highly predictive of the likelihood of pathology on postnatal evaluation [110].

Prenatal US can detect children with a variety of other pathologic conditions, including posterior urethral valves, multicystic dysplastic kidney, VUR, megaureter, duplex anomalies of kidneys with upper pole hydronephrosis, single kidney, polycystic kidney disease, and Eagle-Barrett syndrome [111]. While fetal US remains the primary means of *in utero* imaging, fetal MRI has a role in further characterization of complex fetal genitourinary abnormalities. The pregnant patient is most frequently scanned in the supine position during the second or third trimester. IV contrast material is not used for the exam. The most commonly encountered GU abnormalities on fetal MRI include both upper urinary tract obstruction (UPJ stenosis) and lower urinary tract obstruction such as posterior urethral valves (Fig. 14). More complex

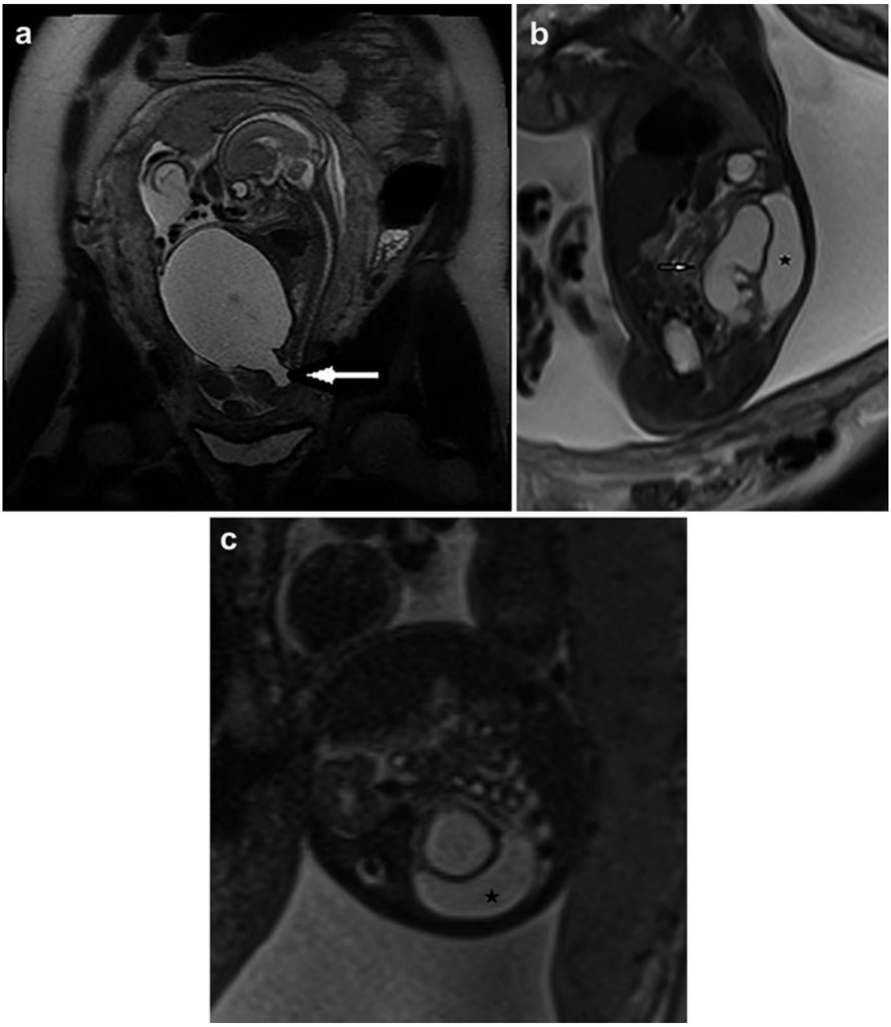


Fig. 14 (**a**) Sagittal T2-weighted fetal MR image of 28-week fetus with dilated bladder and posterior urethra (arrow) in the setting of posterior urethral valves. (**b**) Coronal and (**c**) axial images of a 26-week fetus with left ureteropelvic junction stenosis (arrow) resulting in perforation and retroperitoneal urinoma formation (star)

abnormalities such as cloacal malformations and bladder exstrophy can also be observed with fetal MRI [112].

Megaureter

Megaureter may be primary or secondary to VUR or obstruction. VCUG permits identification of VUR, and diuretic scintigraphy establishes the presence or absence of obstruction. MRU provides excellent anatomic detail, often allowing identification of the site of obstruction [113].

Ureterocele

Ureteroceles may be demonstrated by US, VCUG, IVP, or MRU (Fig. 15). An everting ureterocele may be confused with a bladder diverticulum on VCUG [114]. Ureteroceles may obstruct the urethra during voiding.

Ureteropelvic Junction Obstruction

UPJ obstruction is one of the most common congenital kidney disorders, and most cases are now

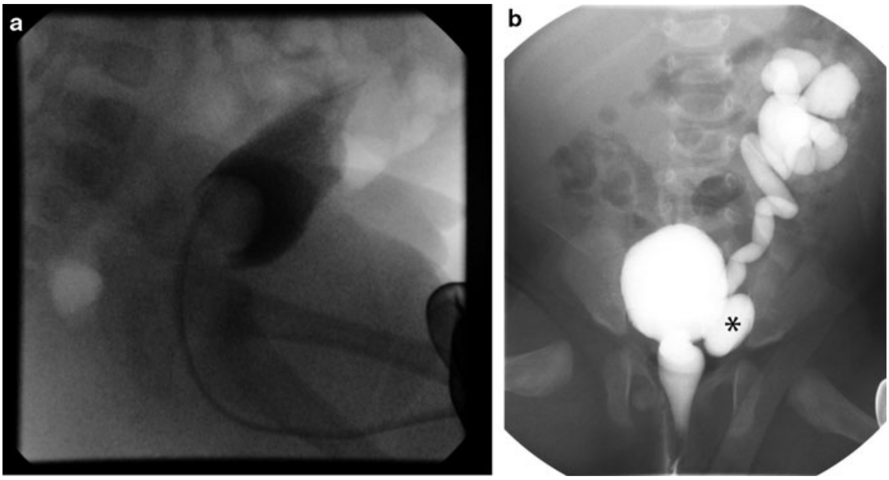


Fig. 15 Ectopic ureterocele with vesicoureteral reflux. (**a**) Voiding cystourethrogram shows rounded filling defect in bladder base from ureterocele. (**b**) Voiding cystourethrogram shows reflux into the lower pole moiety of the left duplex system with eversion of the ureterocele (asterisk). Opacification of the kidney indicates intrarenal reflux

diagnosed by fetal US. Dynamic renal scintigraphy remains the gold standard imaging technique for diagnosing UPJ obstruction (Fig. 16). MRI is an alternative approach that provides functional information and superior anatomic detail without exposure to radiation, albeit with the need for sedation (Fig. 17; [113]).

Abnormal Kidney Number or Location

Unilateral renal aplasia must be distinguished from an ectopic kidney, which can be located anywhere along the course of renal ascent. These ectopic kidneys are often smaller in size. Detection by US is possible, but MRI, IVP, or DMSA is often necessary. IVP may have limited sensitivity if the ectopic kidney has poor renal function.

Horseshoe Kidney and Crossed Fused Ectopia

Horseshoe kidney, with an incidence of 1 of 400 births, is the most common fusion anomaly. Crossed ectopia occurs in an estimated 1 of 1,000–2,000 births. While US can be diagnostic, additional imaging, including IVP, MRI, CT, or DMSA, is often necessary to establish the diagnosis (Figs.18 and 19).

Renal Dysplasia

Renal dysplasia is a histological diagnosis characterized by primitive ducts and cartilage. US evaluation demonstrates increased echogenicity; most dysplastic kidneys are decreased in size. US may detect cysts, which may vary in size and number. The incidence of multicystic dysplastic kidney (MCDK) is estimated at 1 in 2,500 newborn [115]; it is one of the common prenatally detected fetal anomalies. Postnatal US shows cysts of varying size that do not appear to communicate with each other or the collecting system and a small amount of abnormal-appearing renal parenchyma. It may be difficult to differentiate MCDK from severe hydronephrosis [116]. In MCDK, there is typically no communication between cysts, and the larger cysts are not medial (Fig. 20). In hydronephrosis, the calices extend outward from the dilated renal pelvis, and there is functional renal

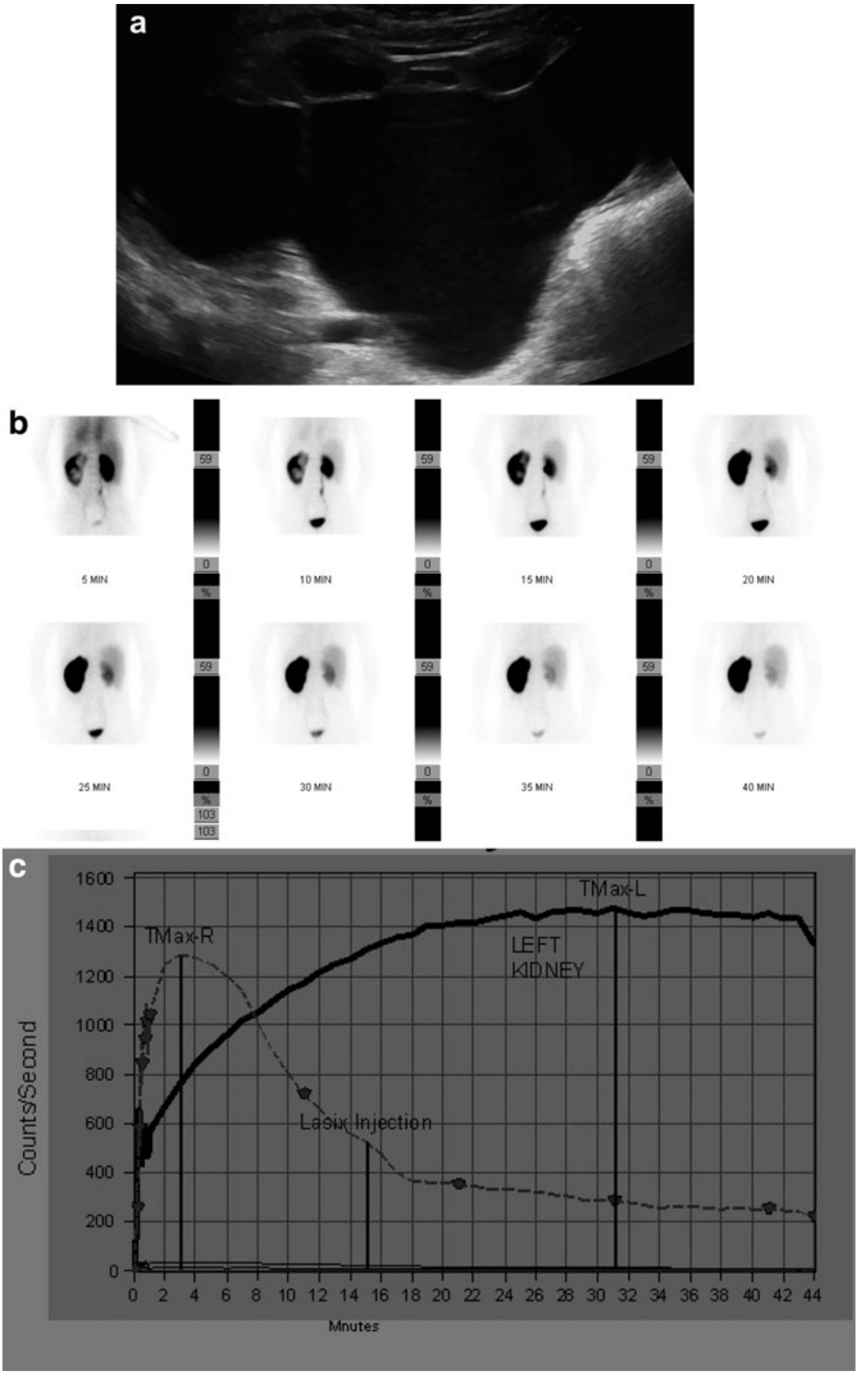

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Fig. 16 Left ureteropelvic junction obstruction. (**a**) US image of left kidney shows dilatation of renal pelvis and calices. (**b**) Posterior images from a ^{99m}Tc-MAG$_3$ renal scan shows retention of radiotracer in left kidney. (**c**) Time-activity curve from ^{99m}Tc-MAG$_3$ scan shows a normal right kidney. On the left, little drainage occurs in response to furosemide. T ½ exceeds 20 min

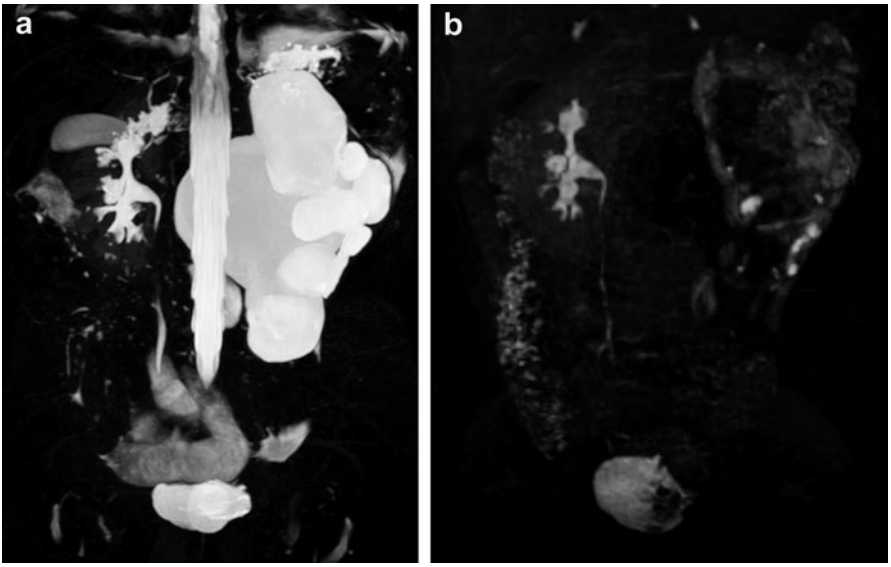


Fig. 17 MR Urography with left ureteropelvic junction obstruction. (**a**) Coronal 3d T2 maximum intensity projection with marked left pelvocaliectasis without ureterectasis. (**b**) Coronal post-contrast 3D T1 maximum intensity projection with delayed excretion on the left

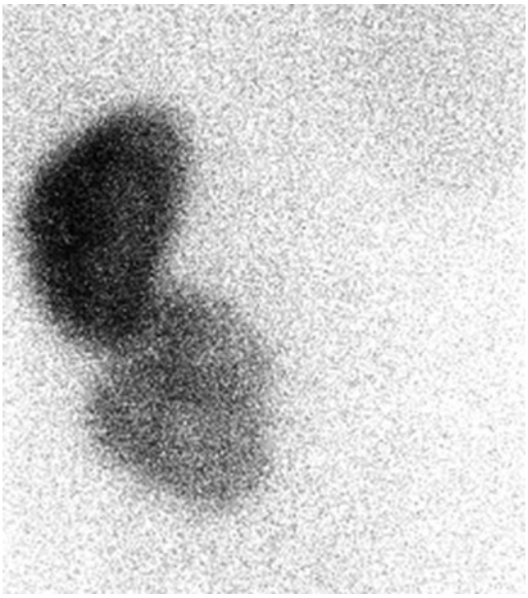


Fig. 18 Crossed fused ectopia. DMSA renal scan anterior image demonstrates a single fused renal moiety on the right

parenchyma surrounding the central cystic structure. If the diagnosis is unclear, a DMSA shows uptake of tracer if hydronephrosis is present, but usually no uptake with MCDK (Fig. 21). Alternatively, MRI can distinguish these two entities.

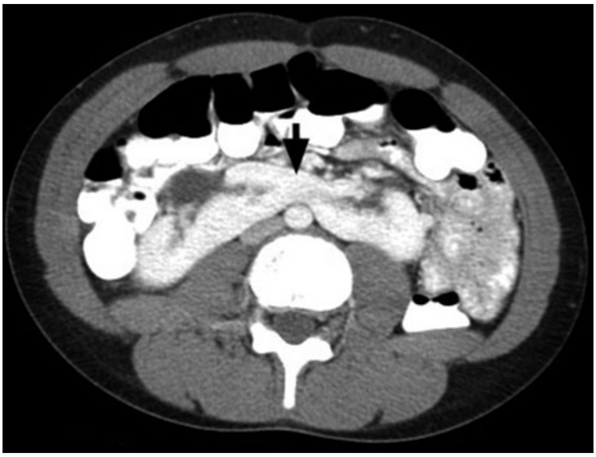
Duplex Collecting System

A duplex collecting system is a common incidental finding and usually of limited clinical significance unless hydronephrosis is also present. Associated anomalies include ureterocele, VUR, ectopic ureters, renal dysplasia, and UPJ obstruction (Figs. 22 and 23). The upper pole is more likely to be associated with obstruction at the distal ureter, while the lower pole is often associated with VUR or UPJ obstruction. US can usually demonstrate division of the kidney but is not reliable in distinguishing between a bifid ureter and complete ureteral duplication. MRU delineates the anatomy of the ureteral duplication when such information is necessary for planning surgery [117]. MRU is especially useful for characterization of the distal ureter. VCUG may demonstrate VUR or ureterocele.

Ectopic Ureters

Ectopic ureters are three times more common in girls, and approximately 80 % are associated with a duplicated collecting system and ectopic insertion of the upper pole ureter. Greater distance from the

Fig. 19 Horseshoe kidney. CT scan of abdomen shows a horseshoe kidney with a bridge of renal tissue fused across the midline (arrow)



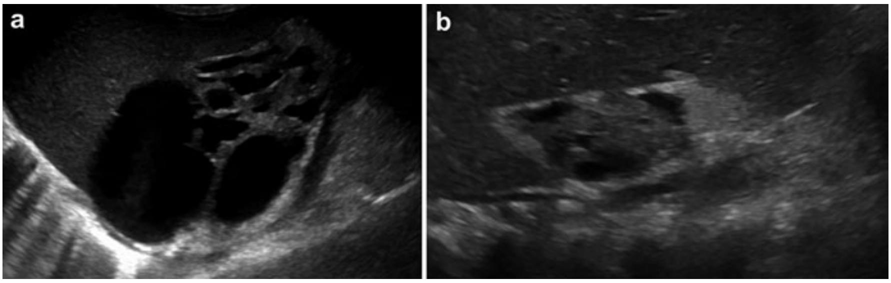


Fig. 20 Right multicystic dysplastic kidney. (**a**) Renal US in a newborn shows multiple noncommunication cysts in the left kidney. (**b**) 3-month follow-up scan demonstrates marked involution of the MCDK

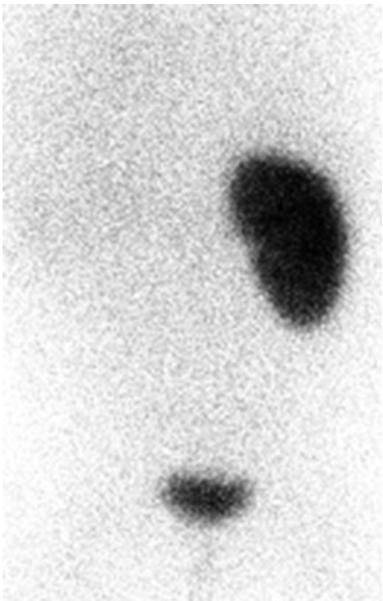


Fig. 21 Left multicystic dysplastic kidney. DMSA renal scan (posterior image) shows no uptake in the left multicystic dysplastic kidney

normal ureter insertion site is associated with more severe ipsilateral renal abnormalities [118]. US can demonstrate duplication, hydronephrosis, megaureter, and abnormal renal parenchyma. MRU often allows detection of the ectopic ureter [113]. VCUG is useful for showing reflux and may indicate the insertion point of the ureter if it is proximal to the external sphincter. DMSA is useful for identifying an ectopic kidney that is the source of an ectopic ureter in a patient with unexplained incontinence [91]. By indicating relative renal function, DMSA may also help with surgical planning.

Bladder Abnormalities

US and VCUG are the standard imaging modalities for patients with a neurogenic bladder (Fig. 24). VCUG readily identifies bladder diverticula, which are easily missed by US. Bladder

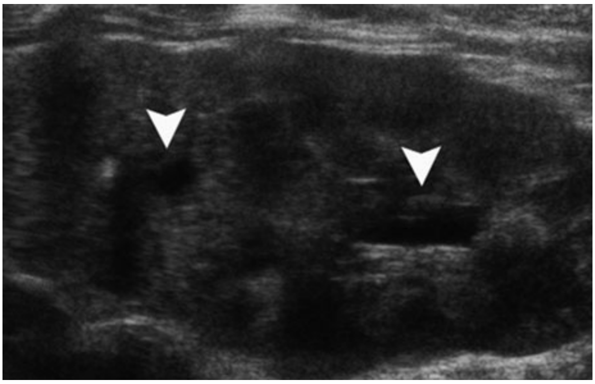


Fig. 22 Duplex system with ectopic ureterocele. Renal US shows mild hydronephrosis (arrowheads) of the upper and lower poles. A band of renal tissue extends across the midpole, indicating a duplex system

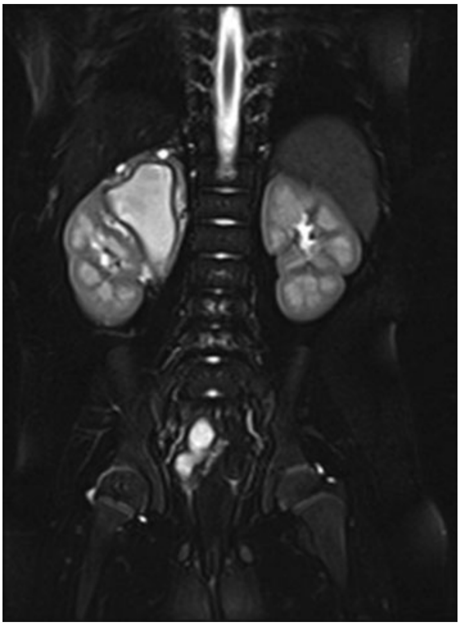


Fig. 23 Duplex system with ectopic ureterocele. MRU shows hydronephrosis of the right upper pole

wall thickening, which is consistent with high bladder pressures, can be quantified by US.

Urethral Abnormalities

VCUG is the definitive test for diagnosing posterior urethral valves (Fig. 25). The prostatic urethra is dilated, the membranous urethra is stenotic, and the distal urethra appears normal. Secondary VUR, hydronephrosis, and bladder abnormalities are often present (Fig. 26). Other urethral abnormalities (anterior urethral valves, urethral duplication, Cowper's duct cyst, urethral diverticulum, megalourethra) are also visualized well via VCUG.

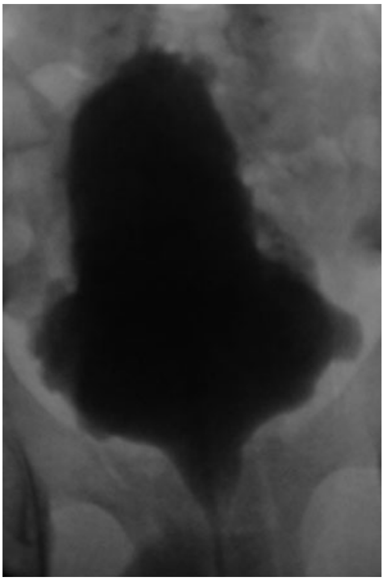


Fig. 24 Neurogenic bladder in a 16-year-old with a spinal cord injury. Voiding cystourethrogram shows pear-shaped bladder with trabeculations

Vesicoureteral Reflux

VCUG is the gold standard for diagnosing VUR, with reflux grades based on the International Reflux Study in Children (Fig. 27) [51]. In clinical situations where the purpose of the study is solely to assess the patient for VUR, RNC and CEVUS are appropriate substitutes for VCUG because of decreased or no radiation burden, respectively.

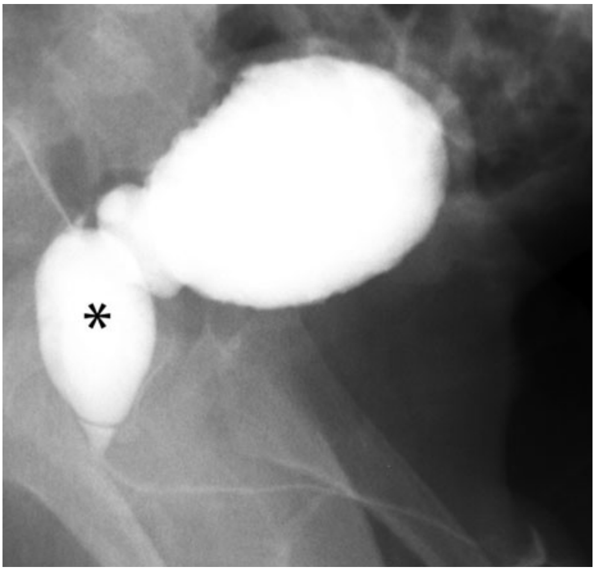


Fig. 25 Voiding cystourethrogram shows dilatation of the posterior urethra (asterisk) in a boy with posterior urethral valves

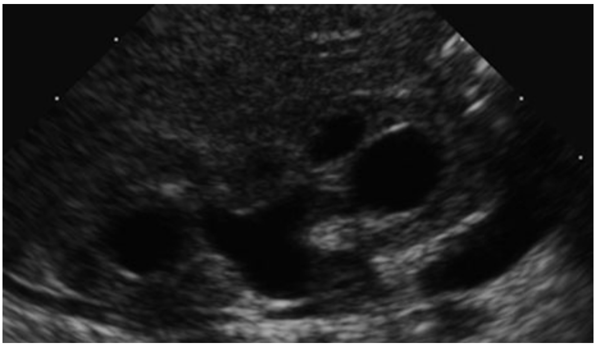


Fig. 26 Renal US shows dilatation of the right renal pelvis, calices, and ureter in a boy with posterior urethral valves

Examples include follow-up studies in children with previously diagnosed VUR, and potentially for the initial evaluation of girls with urinary tract infections (where there are no risks of posterior urethral valves). Renal US lacks adequate sensitivity and specificity for diagnosing VUR [119, 120], although high-grade VUR is more likely if hydronephrosis is present [121].

Hereditary Disorders

Cystic Kidney Diseases

In autosomal recessive polycystic kidney disease (ARPKD), intravenous pyelogram (IVP) shows enlarged kidneys with a delayed nephrogram [122]. Radial streaks due to contrast in the dilated collecting ducts are usually present in infancy but may not be visible in older children. Because of concerns regarding IV contrast, a renal US is now the usual initial diagnostic test (Fig. 28). US shows enlarged kidneys with increased echogenicity and poor corticomedullary differentiation; a hypoechoic rim is often visible [123]. Older children have increased medullary echogenicity, which may resemble nephrocalcinosis [124]. Multiple small cysts may or may not be visible by US [124, 125]. Macrocysts are sometimes visible in older children [125].

In autosomal dominant polycystic kidney disease (ADPKD), US, CT, and MRI are useful for detecting macroscopic cysts and associated complications (Figs. 29 and 30) [126]. The number and size of cysts in children increases with age, and those patients with more cysts have increased

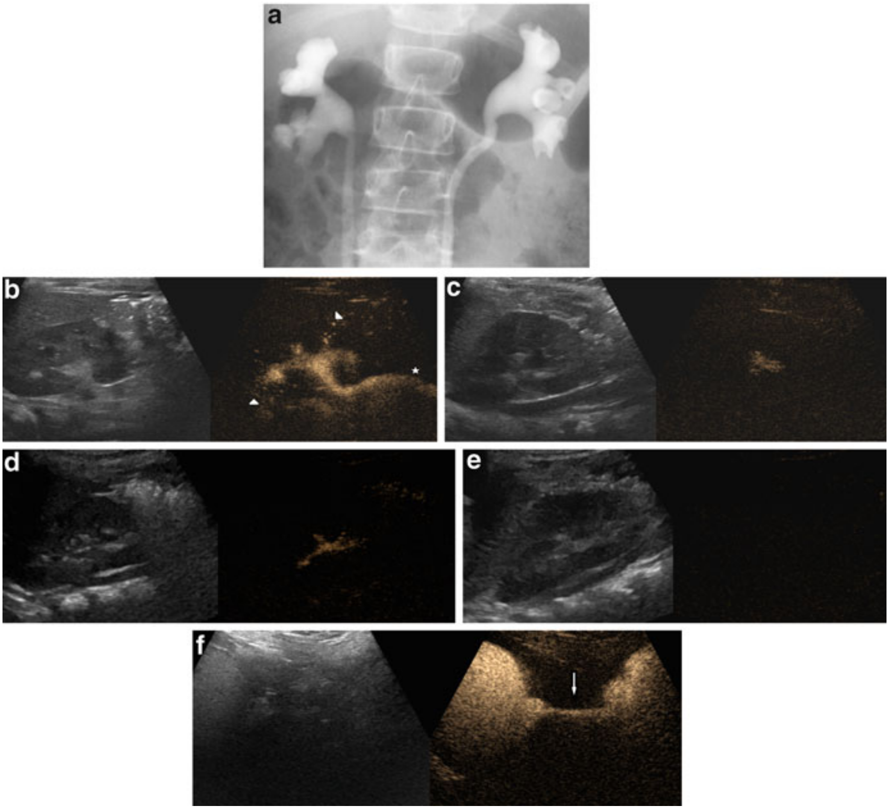


Fig. 27 Vesicoureteral reflux. (**a**) Voiding cystourethrogram shows bilateral grade 4 vesicoureteral reflux. 6-month follow-up longitudinal CEVUS images of the right (**b**) and left (**c**) kidneys show improved reflux on the left with persistent grade 4 reflux on the right, with intrarenal reflux (arrowheads). 18-month follow-up CEVUS images on the right (**d**) and left (**e**) kidneys with grade 2 reflux on the right and resolved reflux on the left. F: Imaging with voiding demonstrates a normal urethra (arrow)

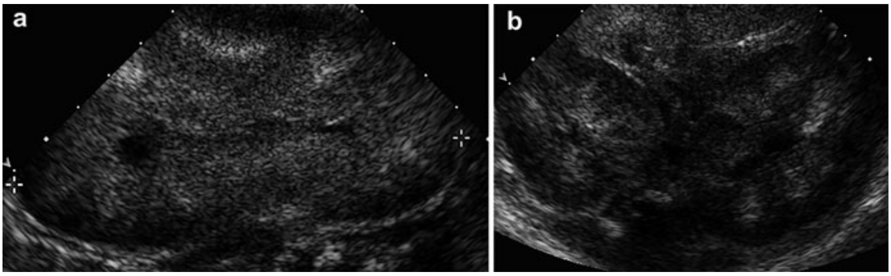


Fig. 28 Autosomal recessive polycystic kidney disease in an infant. (**a**) Longitudinal view shows diffuse enlargement of the right kidney, which is in increased in echogenicity. (**b**) Transverse image through the midpole of both kidneys

kidney size [127, 128]. ADPKD rarely causes renal failure in childhood [37, 129]. In adults, kidney size measured by MRI predicts decline in renal function [126]. Increased renal echogenicity may be seen in some children [125]. Children occasionally have marked disease asymmetry, which can create diagnostic confusion [130]. Both ARPKD and ADPKD can have associated liver abnormalities [131]. Extrarenal complications of ADPKD include liver cyst infection/

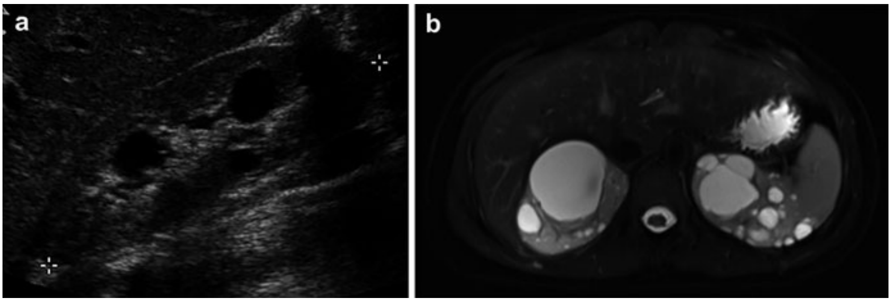


Fig. 29 Autosomal dominant polycystic kidney disease. (**a**) Renal US, longitudinal image, bilateral renal cysts of varying sizes in a 15-year-old. (**b**) MRI, axial fat-saturated T2 weighted image with multiple bilateral T2 hyperintense renal cysts

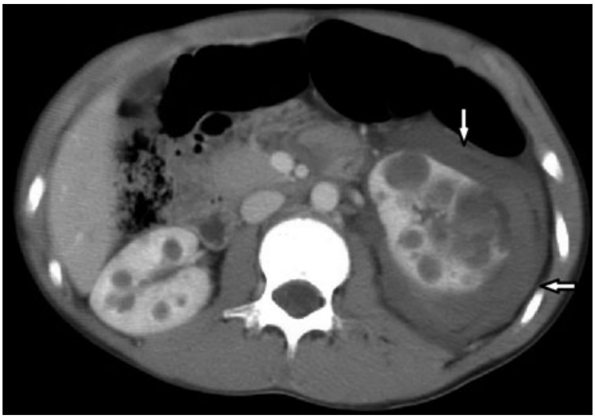


Fig. 30 CT in a 10-year-old with autosomal dominant polycystic kidney disease and bilateral cysts of varying sizes. Left kidney complicated by cyst hemorrhage and perinephric hematoma (arrows)

hemorrhage, intracranial aneurysms, and valvular heart disease [126].

US in nephronophthisis shows hyperechoic kidneys with loss of corticomedullary differentiation and that are of normal or slightly decreased size. Medullary cysts are a hallmark of the disease, although they are not always detected by US [132]. CT and MRI are a more sensitive methods for identifying medullary cysts [133]. The diagnosis of medullary sponge kidney is classically based on characteristic IVP changes showing stagnation of contrast in one or more renal papillae due to dilation of the collecting ducts. The resultant image has been described as a “pyramidal blush.” CT is an alternative approach given the decline in IVPs and may expose patients to less radiation [134, 135]. In medullary cystic disease, the kidneys are usually normal or small, and US or CT may show a few medullary cysts [136].

Tuberous Sclerosis

Angiomyolipomas are the most common renal lesion in tuberous sclerosis (TS) and are readily seen by CT or US (Figs. 31 and 32). Children with disruption of the contiguous *PKD1* and *TSC2* genes usually have severe cystic disease and are at high risk for hypertension and early kidney failure [137]. Renal malignancies, including renal cell carcinoma and malignant angiomyolipomas, are a serious concern in TS, and both are seen in children, sometimes in early childhood [138, 139]. All children with TS should have a renal US at diagnosis and follow-up renal US testing every 1–3 years, with frequency dictated by the specific clinical situation [140]. Patients with extensive or rapidly changing lesions require more frequent follow-up. Those with more severe kidney disease may require CT or MRI to screen for malignant changes.

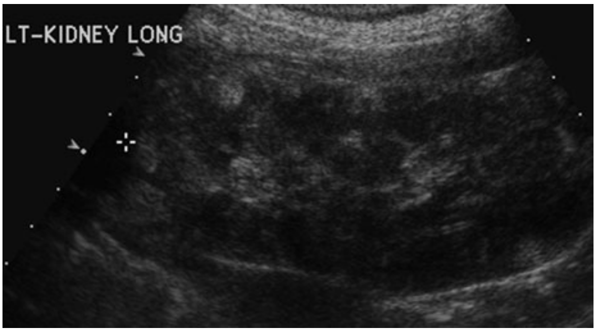


Fig. 31 Angiomyolipomas in tuberous sclerosis. Renal US, longitudinal image, demonstrates multiple small echogenic lesions throughout the kidney. The increased echogenicity is related to intralesional macroscopic lipid

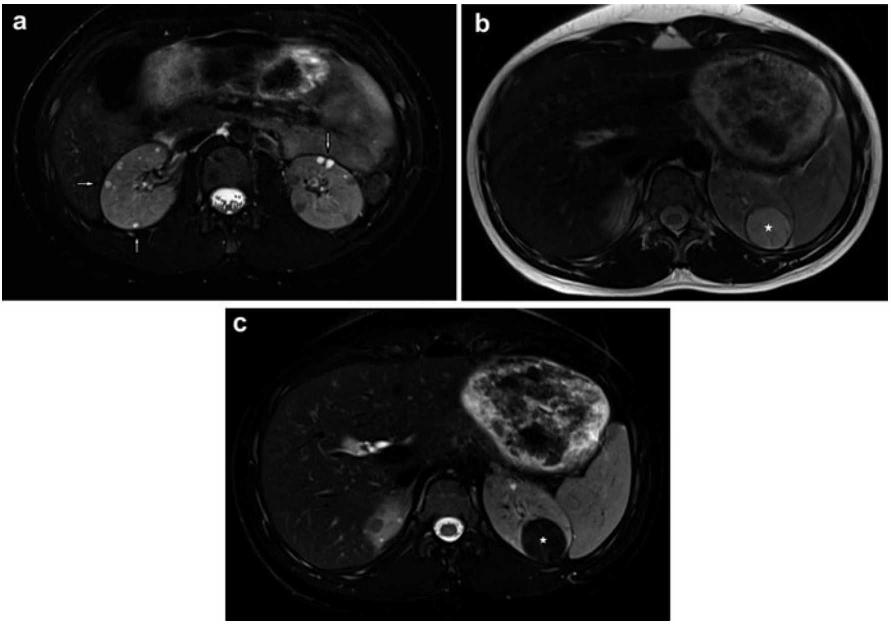


Fig. 32 Tuberous sclerosis on MRI. (**a**) Axial T2 fat saturated T2 weighted image with multiple small bilateral T2 hyperintense cysts (arrows). Axial T2 weighted images without (**b**) and with (**c**) fat suppression demonstrated an angiomyolipoma in the upper pole of the left kidney (star)

Differentiating angiomyolipomas from malignancy requires careful comparison of sequential images.

Infections of the Urinary Tract

Clinical and laboratory criteria do not reliably differentiate pyelonephritis from cystitis [141–143]. DMSA is the gold standard for diagnosing acute pyelonephritis, although MRI and CT provide similar sensitivity [143]. US may detect loss of corticomedullary differentiation and renal enlargement, but lacks adequate sensitivity and specificity when compared to CT (Fig. 33) or DMSA [144–147]. The decreased uptake using DMSA with pyelonephritis may be focal, multifocal, or diffuse. Acute pyelonephritis must be differentiated from renal scarring, which is associated with sharper borders and loss of cortical volume. DMSA may allow diagnosis of pyelonephritis in children who have received antibiotics prior to urine culture or who have a negative culture despite clinical and laboratory evidence suggesting pyelonephritis [148].

DMSA is the current standard modality for diagnosing renal scarring (Fig. 34), with increased

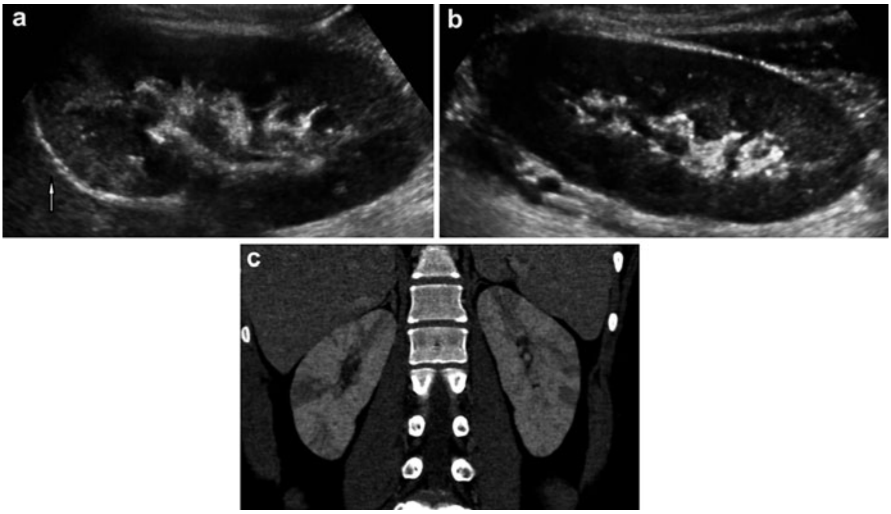


Fig. 33 Acute pyelonephritis. Prone longitudinal images of the left (**a**) and right (**b**) kidneys of a 12-year-old female demonstrate a wedge-shaped region of increased echogenicity in the upper pole of the left kidney. (**c**) Coronal post-contrast CT image of a 10-year-old female with bilateral nephrographic striations, characteristic of acute pyelonephritis

sensitivity when compared to US. Scars appear as photopenic areas with volume loss. DMSA should be deferred at least 6 months after an acute infection to differentiate a transient lesion due to infection from a scar [90, 149, 150]. MRI has the potential to differentiate acute pyelonephritis from renal scarring, which may avoid the need for radiation exposure and obviating the need for two diagnostic tests [151].

Renal Failure

US is the initial imaging modality when a patient presents with unexplained renal failure. Imaging must include assessment of renal size, echogenicity, morphology, cystic disease, and hydronephrosis [3]. US generally allows differentiation of chronic kidney disease (small, echogenic kidneys with a thin cortex) from acute kidney injury. In the pediatric population, the most common cause of end-stage kidney disease is congenital anomalies of the kidneys and urinary tract (CAKUT). This term encompasses abnormalities of the urinary tract (upper and lower tract obstruction as well as VUR), abnormalities related to renal ectopia, and disorders of nephron development (renal cystic disease and dysplasia) [3]. The kidneys may be large if chronic kidney disease is secondary to polycystic kidney disease. US readily detects hydronephrosis, which suggests that renal failure is due to an obstructive etiology. Hydronephrosis may also be secondary to VUR, papillary necrosis, or high urine output; such hydronephrosis is rarely severe.

Renal parenchymal disease is often associated with increased echogenicity, although a normal US examination does not exclude this possibility. The kidneys generally have a normal appearance in prerenal azotemia. The kidneys may be normal or have increased echogenicity in acute tubular necrosis. Echogenic kidneys, which may be moderately enlarged, are often seen in glomerular disease (Fig. 35) [152, 153]. The kidneys are enlarged and very echogenic in HIV nephropathy [154]. The US findings in renal parenchymal disease are rarely specific.

Renal Transplant

US is the primary imaging modality for evaluation of the renal transplant [32, 155, 156]. US can identify posttransplant complications, including

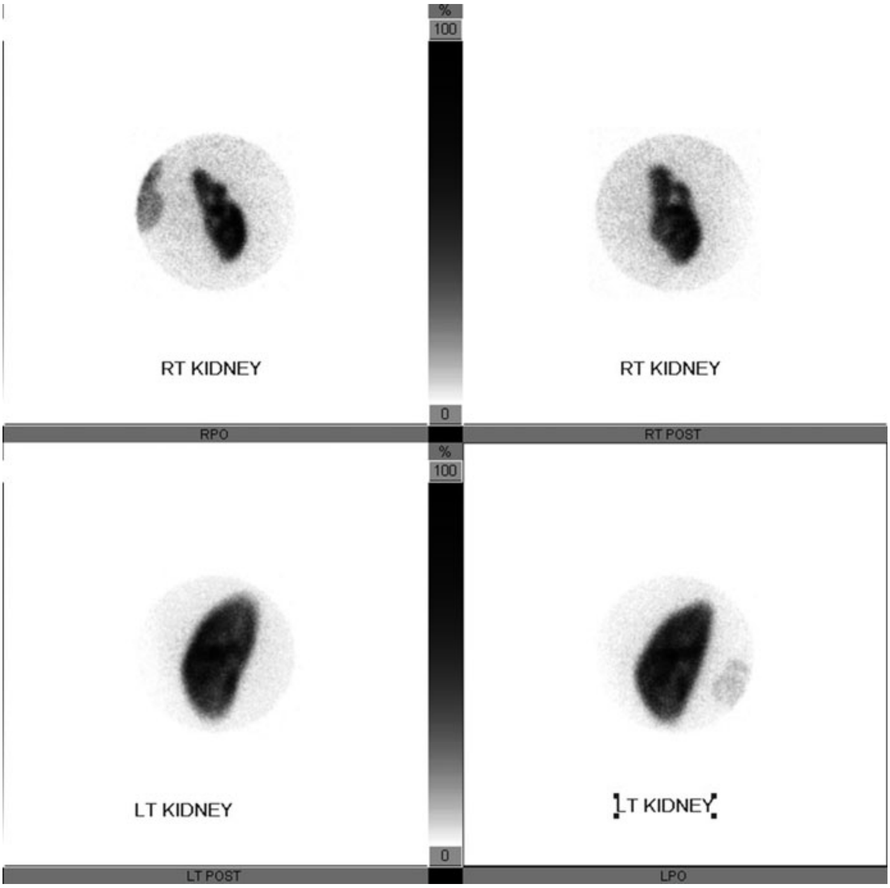
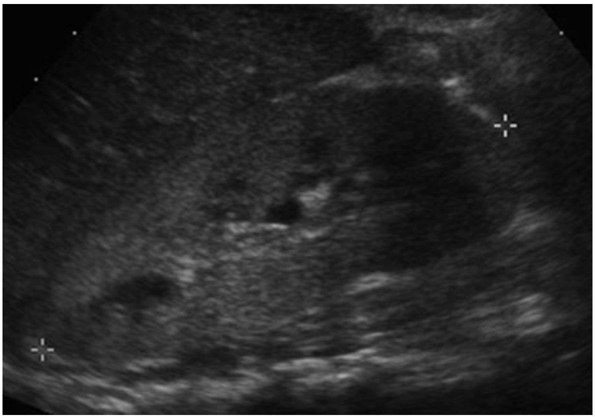


Fig. 34 Renal scarring in a 10-year-old with vesicoureteral reflux. DMSA renal scan shows a small right kidney with diffuse renal scarring. Uptake in the right kidney is 20%

Fig. 35 Acute glomerulonephritis in a 3-year-old. Renal US shows large echogenic right kidney



abnormal fluid collections (e.g., lymphoceles, urinomas; Fig. 36), obstruction, and mass lesions. Doppler US can detect vascular disease after kidney transplant (renal artery thrombosis, renal artery stenosis, renal vein thrombosis, arteriovenous fistula) [155, 156]. Patients with acute graft rejection may have many US abnormalities (increased renal size and echogenicity, decreased

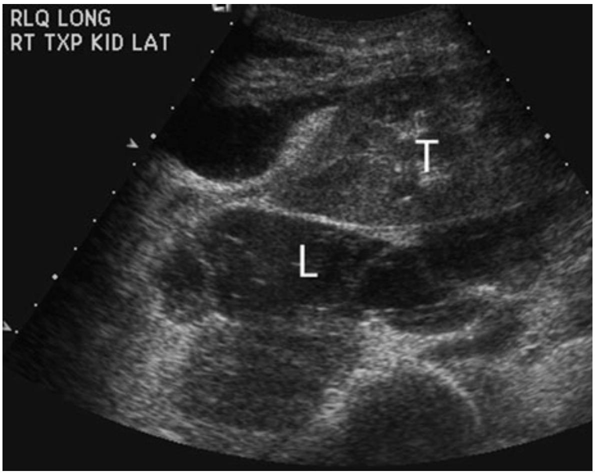


Fig. 36 Lymphocele in a 15-year-old with a renal transplant. Renal US shows a complex, loculated fluid mass adjacent (L) to the transplanted kidney (T)

or absent central sinus echoes); however, US does not have adequate sensitivity or specificity for diagnosis of rejection (for which kidney biopsy remains the gold standard for the diagnosis). Acute rejection, vascular thrombosis, and acute tubular necrosis (ATN) are the most common complications in the immediate postoperative period. Long-term complications include arterial stenosis, ureteral stenosis, chronic rejection, and posttransplant lymphoproliferative disorder [37, 156].

Radionuclide imaging (^{99m}Tc-DTPA or ^{99m}Tc-MAG$_3$) is useful when US does not provide an explanation for graft dysfunction. Absence of flow to the kidney occurs with arterial or venous obstruction or with hyperacute rejection. In contrast, a graft with ATN has normal renal perfusion, but delayed or no excretion. Radionuclide imaging is also useful for demonstrating urinary obstruction or a perinephric fluid collection that is due to a urine leak [157, 158].

Urolithiasis and Nephrocalcinosis

Urolithiasis

US is a sensitive test for detecting calculi in the kidney and the proximal and distal ureter. US does not easily detect stones in the middle portion of the ureter, although hydronephrosis due to obstruction may suggest the diagnosis. Acoustic shadowing is a useful finding but may not be present with smaller calculi. Twinkle artifact, likely secondary to the US beam interacting with stone, can also help identify calculi [37, 159]. US is less sensitive than CT in detecting calculi in symptomatic children (Fig. 37) [160]. CT is also superior to US for identification of bladder stones, especially in patients with bladder augmentations.

Nephrocalcinosis

Nephrocalcinosis is typically detected by US (Fig. 38), although it may be also seen via CT. Plain X-ray only detects nephrocalcinosis in severe cases [161]. Primary hyperoxaluria and cortical necrosis are some of the causes of cortical nephrocalcinosis, while many entities may cause medullary nephrocalcinosis [162].

Trauma to the Urinary Tract

CT with contrast is the primary modality for evaluating a patient with suspected trauma to the kidney (Fig. 39) [163]. CT angiography permits detailed evaluation of the renal artery and vein. US is an alternative imaging modality that may identify large lacerations, hematomas, and urinomas; US with Doppler readily identifies significant injuries of the renal artery or vein. US suggests bladder laceration when there is a large

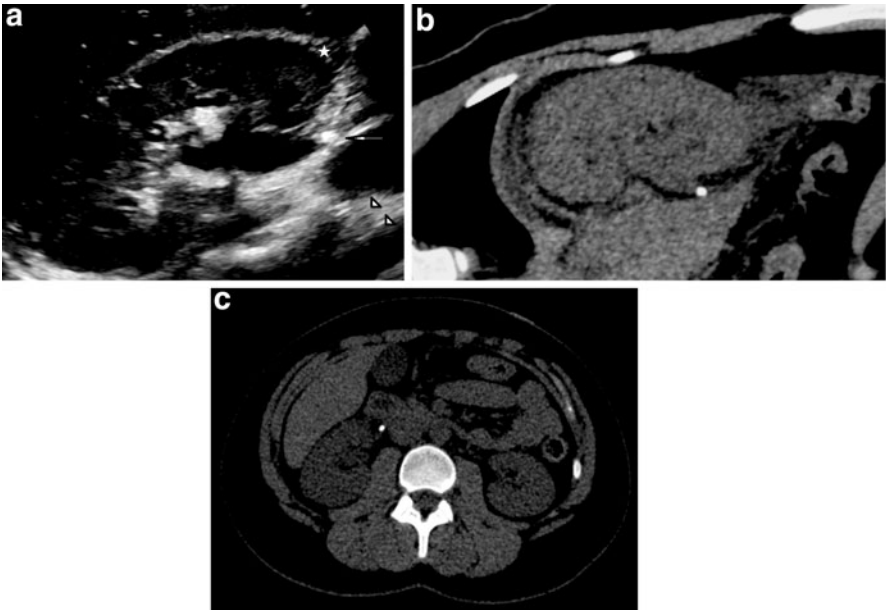


Fig. 37 Nephrolithiasis. (**a**) Longitudinal image of the right kidney with echogenic calculus at the right ureteropelvic junction (arrow) with faint posterior shadowing (arrowheads). Note perinephric edema adjacent to the lower pole (star). Matched plane (**b**) and axial (**c**) non-contrast CT images from same patient

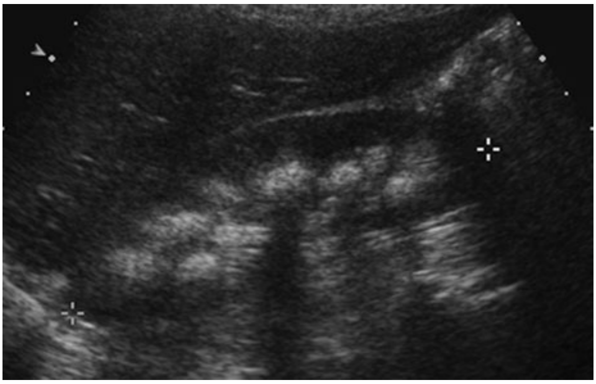


Fig. 38 Medullary nephrocalcinosis in a 12-year-old with renal tubular acidosis. Renal US, longitudinal image, shows echogenic renal pyramids

amount of fluid in the cul-de-sac. When indicated, VCUG can potentially identify the site of bladder leak and detect urethral injury.

Renal and Urinary Tract Tumors

Role of Imaging

US is especially suited for initial evaluation, while CT or MRI is necessary for defining tumor extent and the presence of metastases. CT without contrast allows for identification of tumor calcifications; contrast allows for better differentiation of tumor from normal tissue. US is sensitive for the detection of bladder tumors in children [164].

Imaging of Specific Renal Tumors

Wilms' Tumor

US and CT are complementary in the evaluation and follow-up of Wilms tumor (Figs. 40 and 41) [165, 166]. US with Doppler may provide superior imaging of tumor in the inferior vena cava in some

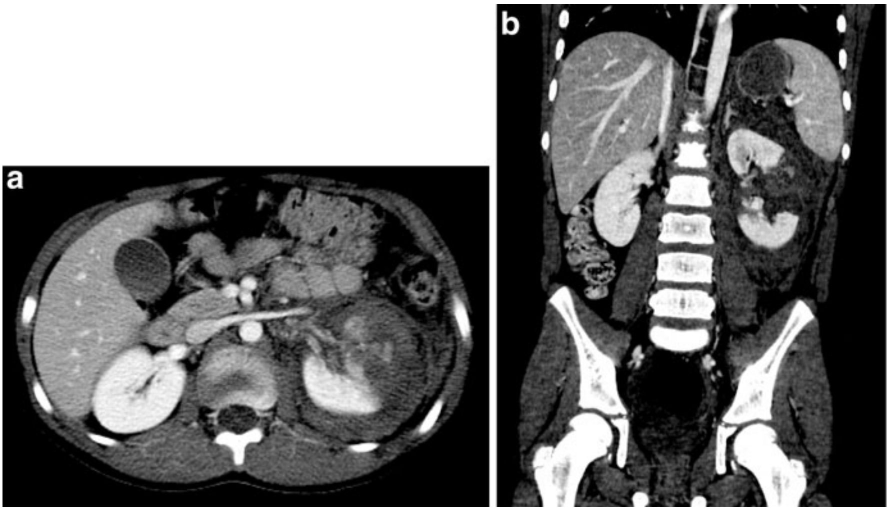


Fig. 39 Motor vehicle collision resulting in extensive left renal laceration with a large perinephric hematoma. A: Axial CT. B: Coronal CT

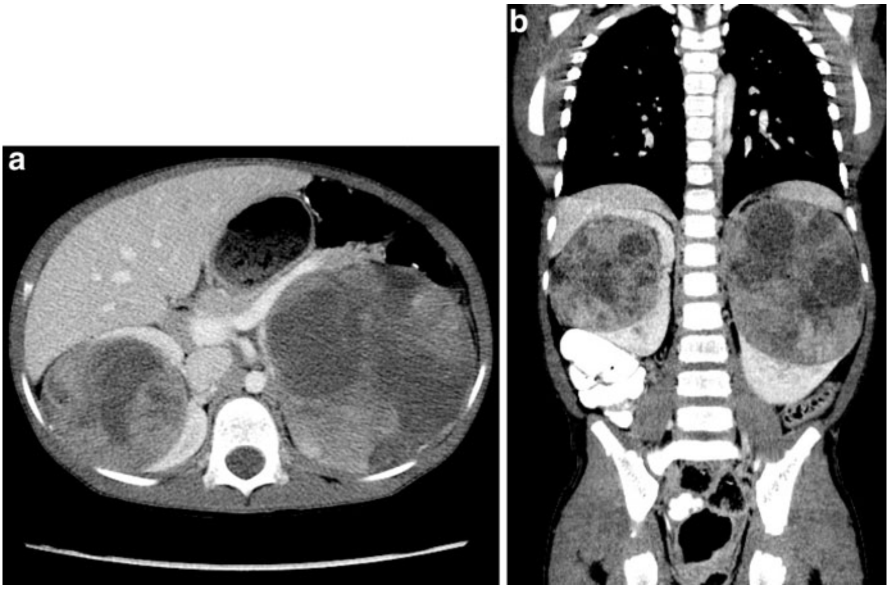


Fig. 40 Bilateral Wilms tumor in a 5-year-old with a palpable abdominal mass. (**a**) Axial CT. (**b**) Coronal CT

patients. Radiologic staging of Wilms tumor includes chest X-ray, US of the abdomen and pelvis, and CT of the chest, abdomen, and pelvis. MRI is also an effective modality for evaluation of Wilms tumor [165, 166]. US is a sensitive approach for screening patients for tumor recurrence, although CT or MRI is necessary to identify nephrogenic rests [165]. US is the preferred modality for Wilms tumor screening, which is necessary in children with syndromes that significantly increase the risk of Wilms tumor (e.g., hemihypertrophy, Denys-Drash syndrome, Beckwith-Wiedemann syndrome) [167]. Screening frequency and duration varies depending on the syndrome.

Lymphoma

Children with lymphoma may have renal involvement. By US, renal lymphoma has decreased echogenicity [168]. CT may demonstrate “contrast inversion” of the lesions in renal lymphoma: increased density compared to normal parenchyma without contrast and decreased density with contrast [168].

Vascular Disease and Hypertension

Renal Artery Stenosis

Doppler US may detect renal artery stenosis in children [169], but sensitivity is limited [170]. US may demonstrate a small affected kidney if the stenosis is severe and long-standing. CT and MRI angiography provide excellent visualization of the extraparenchymal renal artery (Fig. 42), the most

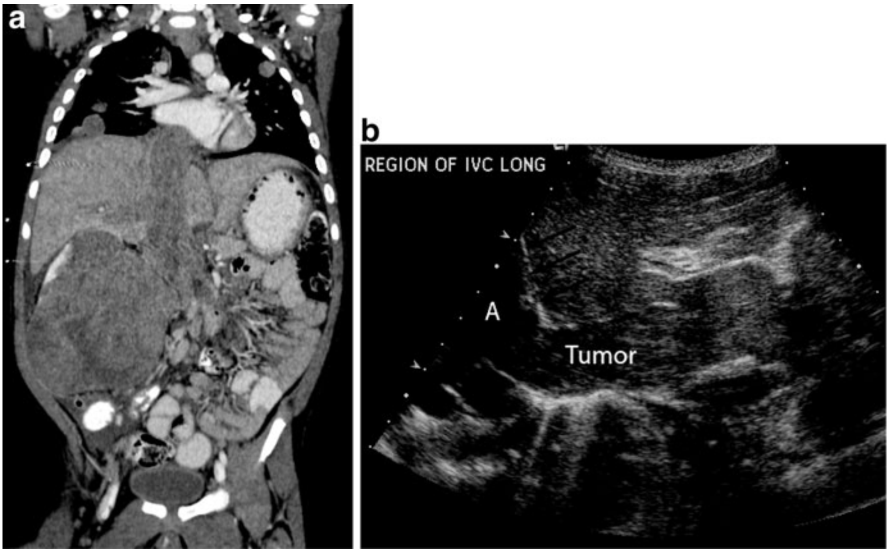
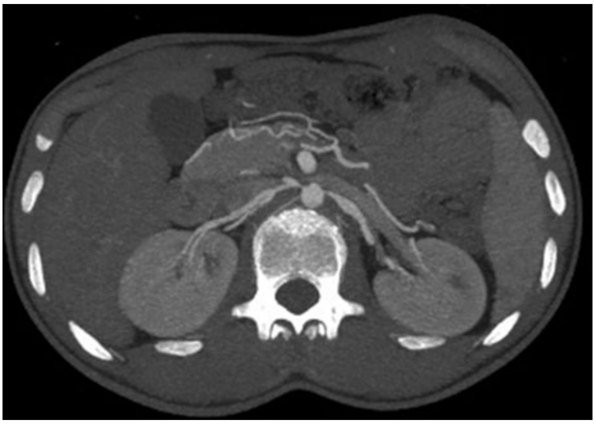


Fig. 41 Wilms tumor in a 2-year-old. (**a**) Coronal CT image shows a large right renal mass, pulmonary nodules, and tumor extension into the IVC to the diaphragm. (**b**) Longitudinal US image with tumor filling the dilated IVC extending to the diaphragm (arrows) and entering the right atrium (**a**)

Fig. 42 Renal artery stenosis in a 13-year-old with neurofibromatosis and hypertension. Axial maximal intensity CT image shows narrowing of both renal arteries near their origins from the aorta



common site of disease, but do not visualize distal branch vessel lesions [171]. When clinical suspicion is high, conventional angiography remains necessary to evaluate children with suspected hypertension due to renal artery disease [170].

Renal Vein Thrombosis

US is utilized to diagnose renal vein thrombosis. The appearance on US varies depending on the timing of imaging. Within the first few days of clot formation, imaging may demonstrate pathognomonic echogenic streaks due to clot in interlobular and interlobar veins [172]. Renal enlargement occurs during the first week and is associated with an echogenic cortex and less echogenic medullary pyramids. Beyond the first week, there is a loss of corticomedullary differentiation, and echogenic streaks are no longer visible. An additional later finding is calcification of thrombi within large or small renal veins [173]. Long-term imaging outcomes vary from a normal-appearing kidney to a small, echogenic kidney with little or no function [173]. In perinatal renal vein thrombosis, renal length is negatively correlated with outcome [174].

US may directly demonstrate clots in the renal vein and inferior vena cava. Doppler US may show absent renal venous flow and pulsatility [172, 173]. The arterial RI is frequently elevated. Clot retraction and formation of collaterals after a few days may create diagnostic confusion due to a return of some venous flow and a decrease in arterial RI. In neonates, adrenal hemorrhage, which is readily seen by US, frequently accompanies renal vein thrombosis and assists in confirming the diagnosis [175]. This association is more common on the left due to the common drainage of the renal vein and adrenal vein.

Pheochromocytoma

Pheochromocytoma may be strongly suspected based on clinical and laboratory evaluation, but imaging is necessary to confirm the diagnosis and allow for staging and resection, which is usually curative. US may identify a pheochromocytoma in or near the adrenal gland, but US lacks the sensitivity of CT or MRI, especially for small lesions or tumors in other locations (Fig. 43). Hypertensive crisis may occur if ionic contrast is utilized, but nonionic contrast appears safe at the doses used for CT [176]. Chest CT or MRI is indicated if an abdominal lesion is not detected or for staging of malignant disease. Pheochromocytomas enhance on contrast CT, although non-enhancing foci of necrosis and hemorrhage may be present. MIBG scintigraphy, a complementary imaging modality, may be appropriate as an initial test, to confirm the diagnosis in equivocal cases or to search for disease when the CT or MRI is negative (Fig. 44) [88, 177]. Positron emission tomography (PET) scan is a potential

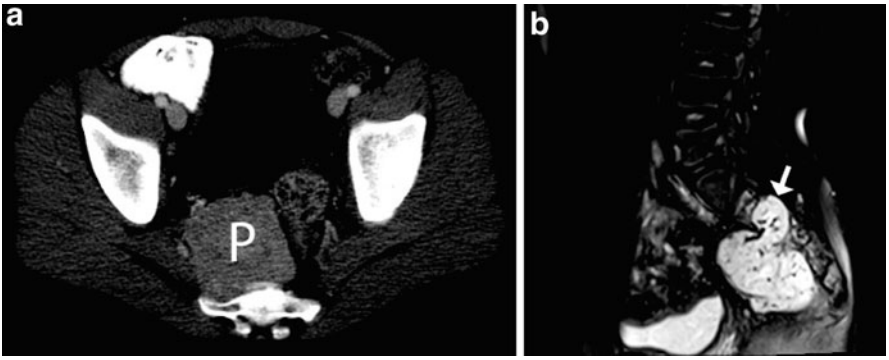


Fig. 43 Pheochromocytoma in a 14-year-old. (**a**) Axial CT image shows a right pelvic mass (P). (**b**) Sagittal T2-weighted MR image shows a right pelvic mass extending into the adjacent neural foramen (arrow)

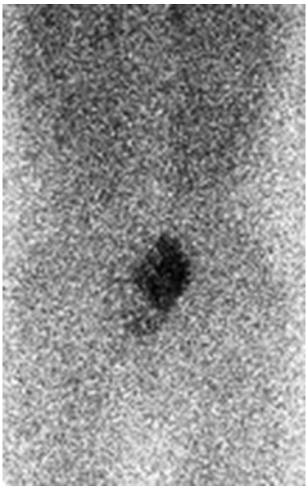


Fig. 44 Pheochromocytoma. Posterior ^{123}I-MIBG image shows uptake into the pelvic tumor

alternative if all other studies are negative and clinical suspicion remains high [178, 179].

Cross-References

- ▶ Autosomal Recessive Polycystic Kidney Disease
- ▶ Pediatric Obstructive Uropathy
- ▶ Pediatric Renal Tumors
- ▶ Renal Transplantation: Surgical and Perioperative Management
- ▶ Urinary Tract Infections in Children

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Technical Aspects of Renal Pathology

7

Patrick D. Walker, Jan U. Becker, and Agnes B. Fogo

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Abstract

This chapter reviews technical aspects and different methods of obtaining kidney biopsy material and allocation of the biopsy specimens. We describe the types of microscopy used to analyze the tissue, including light microscopy with special stains, immunofluorescence microscopy, including salvage techniques from paraffin tissue, and electron microscopy. We describe normal morphology and common, classic morphologic patterns of injury. We also describe selected specific patterns of injury, including mesangial versus endocapillary hypercellularity, membranoproliferative pattern, exudative pattern, crescents, segmental sclerosis, thrombosis, acute tubular injury, interstitial fibrosis/tubular atrophy, and acute interstitial nephritis. Lastly, prognostic implications of biopsy findings and new methods on the horizon are briefly discussed.

Keywords

Biopsy · Biopsy complications · Tissue allotment · Light microscopy · Immunofluorescence · Electron microscopy · Paraffin immunofluorescence · Mesangial hypercellularity · Endocapillary hypercellularity · Crescents

Introduction

This chapter reviews the methods of obtaining the biopsy material and analyzing the tissue and considerations in allocation of biopsy tissue. Normal morphology and examples of classic morphologic patterns of injury are described. Last, experimental techniques that may provide important pathogenic, prognostic, or diagnostic information are discussed.

Obtaining Tissue

General Considerations

Although some renal diseases show diagnostic features by light microscopy (LM), special studies add to the sensitivity of the study. For the renal biopsy to be most useful, it must be evaluated appropriately by an experienced renal pathologist. Biopsies must be examined by specialized LM, immunofluorescence (IF), and electron microscopy (EM) techniques for the most accurate diagnosis [42, 73]. If the nephrologist's hospital does not provide these services, arrangements must be made to send tissue in appropriate fixatives (see below) to a reference laboratory with these capabilities. If such services cannot be provided, it is doubtful whether the institution should be undertaking renal biopsies. Knowledgeable interpretation of light, immunofluorescence, and electron microscopic findings correlated with clinical and laboratory data by a trained renal pathologist in consultation with the treating nephrologist is required for the correct diagnosis [12, 32].

Percutaneous Renal Biopsy

Percutaneous renal biopsy is the most common method for obtaining tissue for the kidney. The technique, first reported in 1951 by Iversen and Brun, historically allowed adequate tissue in more than 95% of biopsies [31, 74]. Blood pressure must be controlled as there is a ten-fold increased risk of complications with blood pressure greater than 140/90 mmHg [42]. Conditions with increased risk of bleeding (e.g., thrombocytopenia, uremia, abnormal INR, bleeding disorders, anticoagulant therapy) should be corrected prior to biopsy. Contraindications to percutaneous biopsy include abnormal renal vascular supply, inability to accurately image the kidney, and uncontrolled hypertension [5, 29]. In the era of ultrasound guidance and automated biopsy instruments, a solitary, ectopic, or horseshoe kidney may be biopsied safely in selected patients [5, 23, 29]. Relative contraindications include obesity, uncooperative patients, hydronephrosis, ascites, pregnancy, and small scarred kidneys, all associated with greater risk for complications. Open or modified open biopsy is preferable if the biopsy information is crucial in these conditions. Percutaneous biopsy is also contraindicated if the kidney has tumors, large cysts, abscesses, or pyelonephritis because the needle track may facilitate spread of malignant cells or infection. Open biopsy allows selection of specific areas for biopsy in these situations.

Food and drink should have been withheld for at least 6 h before biopsy, and the child should be lightly sedated. The patient is prone usually with a sandbag or rolled sheet beneath the abdomen for correct positioning, with surgical sterile preparation of the flank and the lower pole of the kidney located by computed tomography (CT) or ultrasound, the last being the most commonly used method. The left is preferred because in most cases, no other organ hinders access. However, if bowel or splenomegaly blocks access, the other kidney should be visualized, or the procedure halted. Local anesthetic is infiltrated first in the skin and then in deeper tissues, taking care not to enter the kidney, and the needle or trocar introduced. Ultrasound shows the needle as it is advanced towards the kidney in real time. The operator will feel the change in resistance as the needle reaches the kidney and then ultrasound will reveal the angle of approach and needle location relative to the capsule at the lower pole [13, 15, 16, 26, 44]. If the needle has entered the kidney, it should be retracted, slightly redirected, and localized with ultrasound for correct placement (Fig. 1). The speed of automated biopsy needles minimizes the need for the patient to hold respirations, required with conventional needles. Computed tomography (CT)-guided biopsies differ in that the kidney to skin distance is determined and a trocar is placed. CT is repeated to determine the angle of approach and that the trocar is at the kidney capsule.

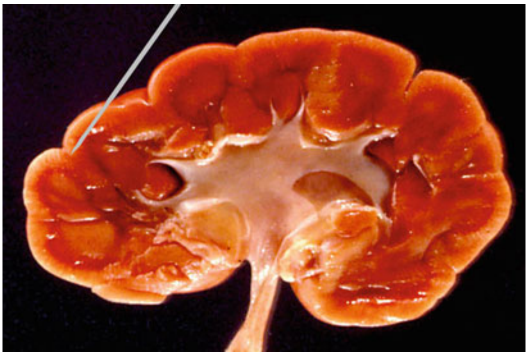


Fig. 1 Cross section of a kidney from a four-year-old boy. Schematic needle enters at the lower pole and captures only cortex because of the correct placement and angle

Needle gauge determines the quality, volume, and glomerular number obtained (Fig. 2). The internal diameter of the 18 g needle (300 μm) is slightly larger than the diameter of a normal glomerulus (200–250 μm, in patients 2 years and older) [17, 67]. As a result, an 18 g needle results in about 50% lost, fragmented, or floating glomeruli (Fig. 3) [71]. It is therefore highly recommended that a 16 g needle is always used in children beginning at age two and the 18 g needle reserved for infants and young toddlers.

The biopsy needle ideally advances through the cortex with little medulla (Fig. 1). Inclusion of the corticomedullary junction in pediatric biopsies is useful because the segmental lesions of focal segmental glomerulosclerosis (FSGS) start in this location in the earliest stages of disease. Two or three passes are planned but the number of passes depends on the patient's status during the procedure and the tissue obtained with each pass. A postbiopsy ultrasound can be done, and, in the absence of active extravasation, the patient is very unlikely to develop a late major hemorrhagic complication. Pressure is applied to the site and a bandage placed. The patient should lay supine in bed for 6 h and may be discharged usually after

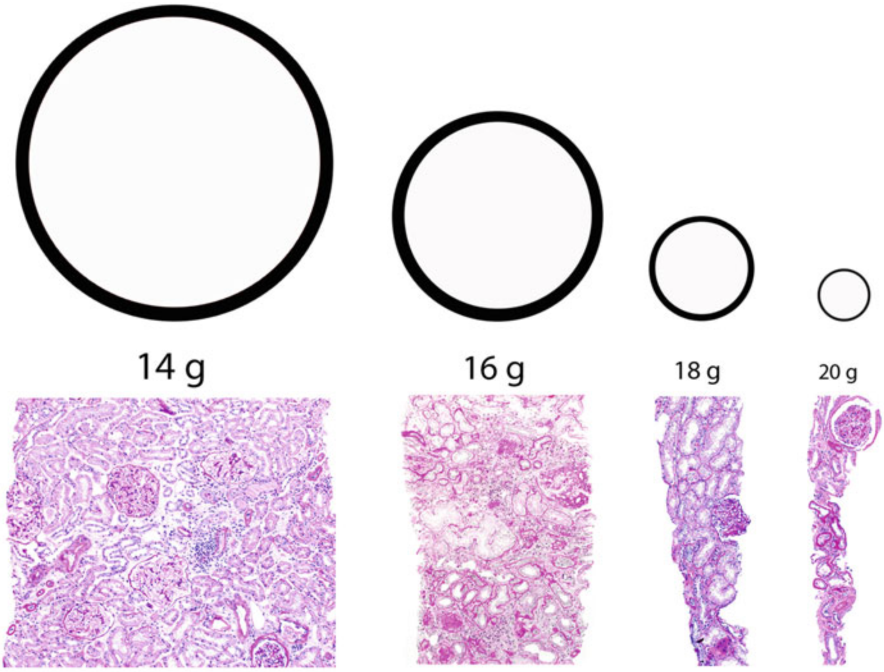


Fig. 2 Needle gauge and tissue width. The typical internal diameter of the various gauges of biopsy needles are shown at the top. Sections of renal biopsy tissue obtained from each gauge are shown below (original magnification 200×). The mean diameter of tissue sections obtained with each needle gauge is shown beneath ($n = 25$ personal communication, PDW). For reference, the diameter of a typical glomerulus from children 2 years and older is 200–250 μm

6–12 h. Vital signs, complete blood counts, and urinalysis for blood are monitored till discharge. The patient is instructed to rest at home for at least the next 24 h. Those with post biopsy perinephric hematomas, postbiopsy gross hematuria, or very young children can be observed overnight so that hemostasis is assured [62, 63, 69].

Complications are recognized within 8 h of the biopsy in most cases [3, 70, 72]. Microscopic hematuria is the most common finding (almost 100%) followed by a hematoma at the biopsy site (>90% in controlled studies) [51], pain at the biopsy site (4%) [50], and macroscopic hematuria 4%. Symptomatic arteriovenous fistula formation occurs in <5%. However, when using renal ultrasound and Doppler mapping, an arteriovenous fistula was found in 14% of patients 24 h after biopsy [66]. None in their series required intervention and all self-closed over the next one to 5 months. Very rarely, arteriovenous fistulae are symptomatic with falling renal function, significant macroscopic hematuria, etc., and these generally require intervention [18, 53]. In systematic reviews, major complications following biopsy occurred in <2% of patients overall including bleeding requiring transfusion (6%), radiologic intervention (0.3%), or nephrectomy (0.01%) [1, 4, 7, 8, 35]. Death due to biopsy occurred in up to 0.06% of all biopsies with 0.03% in outpatient biopsies [50]. Rarely, development of bladder clots can be obstructive and require Foley catheterization and irrigation. Other complications have been reported, including retroperitoneal hemorrhage, inadvertent puncture of other viscera or major renal vessels, sepsis, renal infection, and seeding of cancer. The safety of the biopsy depends upon the correct angle of approach, accurate localization to the lower pole, and placement of the needle on, not through, the capsule. Though perhaps counter-intuitive, the needle gauge plays little or no role in the complication rate in the hands of a trained operator [11, 43]. Over the last decade, as the primary operator has changed from the nephrologist to radiologists in some areas [36], there has

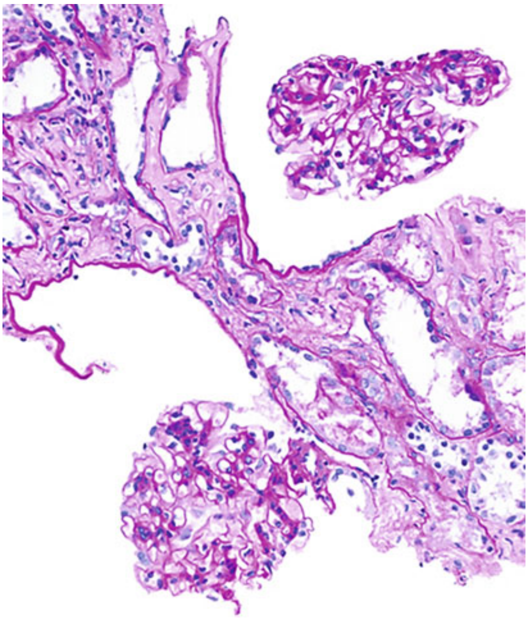


Fig. 3 Typical section of kidney tissue obtained from an 18 g needle biopsy. Bowman's capsule is torn, and the glomeruli are floating (periodic acid-Schiff stain, original magnification 200×)

been a decline in tissue adequacy. Quality improvement efforts in each center can lead to a return to historical norms [21].

Other Biopsy Techniques

Alternatives to percutaneous renal biopsy include open or laparoscopic surgical biopsy and trans-jugular renal biopsy [77]. Likely because the patients requiring these alternatives are at significantly higher risk, the complication rate may in some series be higher than with routine percutaneous biopsy [68]. The surgical biopsy has similar adequacy, but the trans-jugular biopsy is much smaller and fragmented often making analysis difficult [47]. Superficial wedge biopsies from open surgical biopsies may undersample disease-specific lesions and oversample areas of subcapsular scar. Larger, deeper wedges, or needle biopsies should therefore be considered in this setting. The requirement for diagnostic material must significantly outweigh the increased invasiveness of these alternate techniques.

Assessment of the Renal Biopsy

Adequacy of Sample

Cores of fat and connective tissue will float when placed in saline, but a core of renal parenchyma will sink. In diffuse disease, such as membranous glomerulopathy, one glomerulus may be adequate for diagnosis. However, in other diseases, such as crescentic glomerulonephritis, FSGS, or lupus nephritis, diagnostic lesions may be focal. The greater the number of glomeruli sampled, the lower the probability of missing a focally distributed lesion [14]. If only 10% of glomeruli in the kidney are involved by the focal process, a biopsy sample of only 10 glomeruli has a 35% probability of missing the lesion, decreasing to 12% if the biopsy contains 20 glomeruli. When one-fourth of glomeruli are involved in the kidney, there is only a 5% chance of missing the abnormal glomeruli in a biopsy of 10 glomeruli. A biopsy sample of 20–25 glomeruli for light microscopic assessment is sufficient to distinguish between mild disease (less than 20% of glomeruli involved), moderate disease (20–50% of glomeruli involved), and severe disease (more than 50% of glomeruli involved). The sample site must also be considered in evaluating the adequacy of tissue. Even a large biopsy, consisting only of superficial glomeruli, cannot exclude the presence of early FSGS, in which the initial involvement is in the juxtamedullary glomeruli. Likewise, a biopsy containing cortex and juxtamedullary tissue is needed to raise the histopathologic suspicion of nephronophthisis in children and young adults with unexpected chronic kidney disease [40].

Allotment of Tissue

Renal tissue should be studied by light microscopic techniques with special stains (hematoxylin and eosin, modified silver stain [periodic acid/methenamine, also called Jones' stain], periodic acid-Schiff [PAS], and/or Masson trichrome), IF and EM [12, 24, 61, 73]. The tissue is divided so that glomeruli are present in each portion of the sample (Fig. 4). Fixative and transport media should be available in

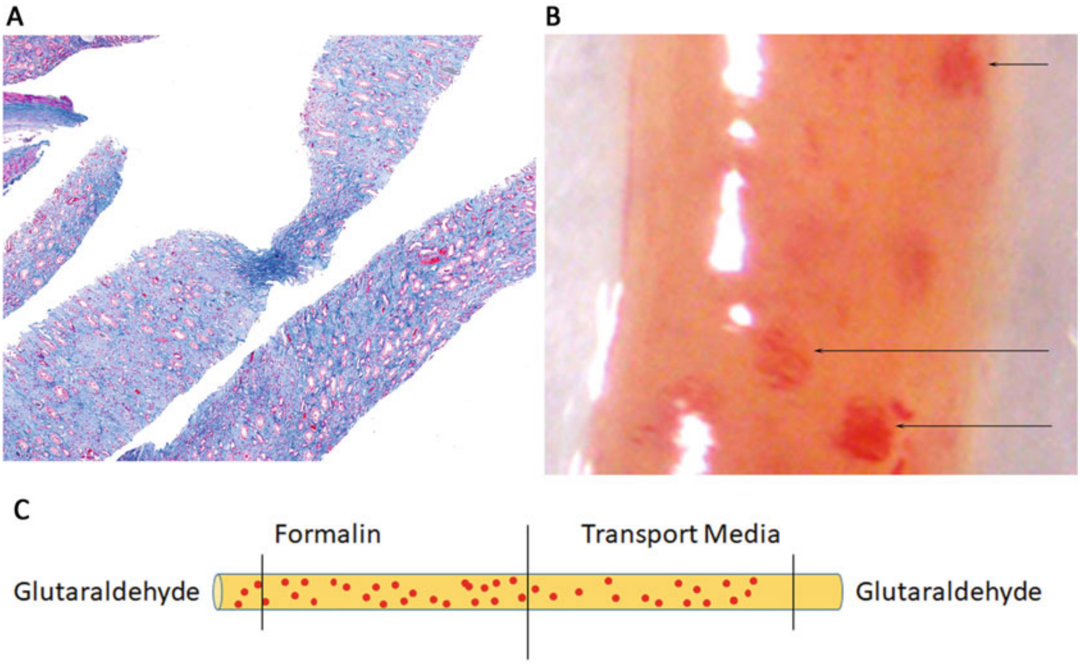


Fig. 4 (**a**) Crush artifact from the use of a forceps to remove the tissue from the biopsy needle (trichrome stain, original magnification 40×). (**b**) Dissecting microscope photograph of a biopsy sample (glomeruli are seen as red balls, arrows, original magnification 15×). (**c**) Schema for optimal allocation of core tissue. Allocate the sample by cutting it with a portion placed in formalin and the remainder in transport media for immunofluorescence (Michel's), after 1 mm cubes are removed from each end and placed in glutaraldehyde (red dots signify glomeruli)

the biopsy suite. The biopsy optimally is timed so that an experienced technician or pathologist can attend to ensure prompt processing of the biopsy tissue. The speed with which the sample is placed in fixative determines the quality of the morphology which can be obtained, particularly for electron microscopy. Thus, each core should be allocated as it is obtained, not waiting until all passes are made. If this is impossible, tissue may be placed in saline and brought directly to the laboratory for processing. After each biopsy pass, the tissue should be carefully removed using a needle or a wooden stick but never a forceps, as the forceps can cause crush artifact (Fig. 4a). The tissue core is places on a clean smooth surface (i.e., wax board) or other nonstick pad (not a gauze pad or sponge, which also causes divoting artifact as the tissue molds to the holes of the underlying surface). The tissue ideally is then examined for the presence of glomeruli using a dissecting microscope or a "smart" phone camera. Glomeruli are identified as red dots and, when present, are identifiable >80% of the time (Fig. 4b). Of note, in severely scarred kidneys or with very low GFR, visualization of glomeruli may be difficult. When a dissecting scope is not available or visualization is not possible, allocation of tissue can then optimally be done by cutting 1 mm pieces off each end of each core for electron microscopy, and dividing each remaining core into two pieces, with the larger portion (about two-thirds of the length of the core) for light microscopy and the remaining small piece for immunofluorescence (Fig. 4c). This approach optimizes chances of glomeruli available for each modality.

When the tissue sample obtained is very small, the nephrologist and the pathologist should consider the differential diagnosis and allocate tissue accordingly. For example, in a case of suspected IgA nephropathy, tissue for IF is most important. IF studies can be attempted on remaining fixed tissue in the paraffin block but are not as reliable as when performed on frozen nonfixed material. Electron microscopic studies can be done on other portions of tissue (as long as mercury-based fixatives have not been used). When tissue for EM is inadequate, portions of the paraffin-embedded tissue left after light microscopic examination may be cut out from the block and processed for EM. Although the quality is not optimal, diagnostic findings can still be discerned. In special circumstances, when no tissue remains in the paraffin block and a focal lesion in one section must be studied, one may attempt to process the tissue section from a glass slide for EM.

Light Microscopy

Numerous fixatives are used for light microscopic examination, and they vary from institute to institute. Satisfactory results may be obtained with Zenker's, Bouin's, or Carnoy's fixatives, formalin, or paraformaldehyde. Material for IF studies may be snap frozen immediately at −20 °C in solutions of isopentane, dry ice, acetone, or Freon and embedded in Tissue-tech, OCT, or other compounds for frozen sections. If tissue cannot immediately be snap frozen, it may be placed in Michel's tissue media, where it may be stored for up to 1 week before freezing. This allows tissue to be sent to reference laboratories for appropriate processing. Tissue for EM may be fixed in glutaraldehyde, formaldehyde, or other appropriate nonmercury fixatives. Tissue placed in glutaraldehyde should be promptly processed, or if stored for future possible processing, should be transferred to an appropriate buffer solution within a week to avoid artifacts. Tissue for LM is routinely processed, embedded in paraffin, and cut into 2- to 3-μ thick sections. Serial sections with multiple levels are then prepared for examination.

If water-soluble compounds are expected, such as urate or uric acid, the tissue should be fixed in ethanol. Lipids are best detected in frozen sections because they are extracted during alcohol and xylene processing for paraffin sections. Hematoxylin and eosin stains are most useful for overall assessment of the interstitium and crystals. This stain also allows particularly good visualization of infiltrating cells, especially eosinophils. In addition, fibrin may be easily visualized by this stain. Additional stains are routinely used for kidney biopsy assessment (Fig. 5). Periodic acid-Schiff (PAS) stains glycoproteins and accentuates basement membranes and matrix material and allows definition of the brush border of proximal tubular cells. Areas of hyalinosis and protein precipitation, including cryoglobulin (which usually is dominantly IgM), are also accentuated with PAS. Silver stains, such as Jones' stain, stain basement membrane material but not cells or deposits, thus allowing distinction of these components. Masson's trichrome stain detects areas of collagen deposition by staining bluish. Sirius red stains all collagens and can also be visualized and quantified under polarized light to assess fibrosis. Other special stains, such as immunohistochemistry for specific molecules, may be indicated. Congo red stain detects amyloid. Special stains can detect bacterial or fungal organisms and acid-fast bacilli. Special techniques may also be used on the light microscopic material. These include polarization to detect crystals or foreign bodies and morphometry to assess glomerular size and severity of interstitial fibrosis quantitatively.

Immunofluorescence Microscopy

Immunofluorescence (IF) studies are most commonly done by direct IF on frozen tissue sections with application of fluorescein-conjugated antibodies directed against IgG, IgA, IgM, complement components C3 and C1q, kappa, lambda, and often albumin and fibrinogen. Additional antisera may be used as clinically indicated. Phospholipase A2 receptor (PLA2R), the antigen in most cases of so-called primary membranous nephropathy (MN), is detected by IF by digestion techniques, followed by applying antibody to PLA2R to tissue section cut from the paraffin block or by IF on frozen tissue [6, 37]. Additional antigens in membranous nephropathy have been identified by mass spectroscopy, and immunofluorescence for immunohistochemistry methods developed for their detection in the deposits of cases of membranous nephropathy [60]. These novel antigens include thrombospondin type 1 domain-containing 7A (THSD7A),

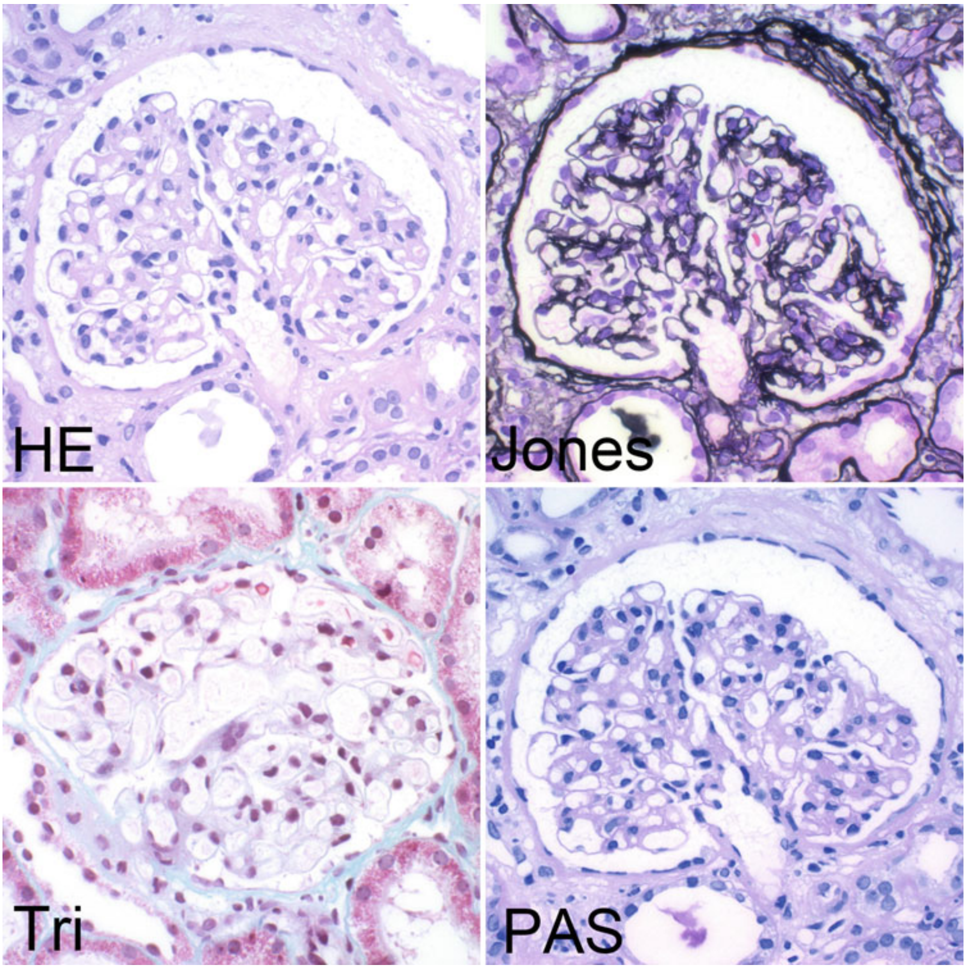


Fig. 5 A normal glomerulus shown with hematoxylin and eosin (H&E), Jones, trichrome (Tri) and periodic-acid Schiff (PAS) staining. These are the principal stains used routinely for the examination of paraffin-embedded tissue in renal pathology (original magnifications ×400)

exostosin-1,2, neural EGF-like-1 NELL-1, and semaphorin 3B (Sema3Band PCDH7) discussed in sections on membranous nephropathy [27, 60].

Additional immunostaining studies include those for hepatitis B antigen, thyroglobulin, C4, C4d, C3d, specific viruses (e.g., SV40 for polyoma virus), myoglobin, and type IV collagen alpha chains. Some of these antigens are recognized by antisera even after fixation and can be detected by immunohistochemical techniques (e.g., immunoperoxidase) on the formalin-fixed tissue sections and then studied with a light microscope. This technique requires enzyme pretreatment of tissue depending on the antigen to be studied, which must be tailored exactly depending on length and type of fixation and section thickness. Direct observation of the digestion process, stopping when all plasma is removed from capillary loops, has been used to achieve reliable results [20]. These challenges have prevented widespread use of this technique for the routine immunostaining panel performed in the USA. In general, IF on frozen tissue for detection of immunoglobulins and complements is more sensitive than IHC or even IF on fixed tissue.

Frozen tissue sections stained by the commonly used method of fluorescein-conjugated antibodies are viewed by IF microscopy, evaluating staining in glomeruli, vessels, tubules, and interstitium. The pattern of glomerular staining is assessed to define granular or linear capillary wall staining or mesangial deposits. Arteriolar staining may be of diagnostic significance to detect, for example, deposits in lupus. Tubules may show granular deposits in lupus nephritis, pseudolinear deposits in light chain deposition disease, or linear staining in the rare anti-TBM antibody disease. Nuclear staining can be seen in lupus or lupus-like diseases as a tissue manifestation of the patient's positive ANA. IF is more sensitive than EM in identifying immune deposits, although EM provides more detailed information on the exact localization of those deposits [24, 61]. Diagnostic lesions of interest should be photographed because fluorescence fades on storage and with light exposure.

If there is no tissue available for IF by frozen section, the paraffin-embedded tissue can be used [46, 49]. This can salvage what would have been a significant biopsy miss. IgA deposits are usually readily detectable by this technique and can allow specific diagnosis of IgA nephropathy in cases where glomeruli are not present in the frozen tissue. In addition, paraffin IF has become a required technique for the correct diagnosis of a specific subset of glomerular diseases in which there may be masked deposits. The finding of a proliferative glomerulonephritis with C3 only with or without a membranoproliferative pattern is consistent with C3 glomerulopathy (C3G, see ▶ Chap. 22, "Membranoproliferative Glomerulonephritis and C3 Glomerulopathy in Children"). However, this is an extremely rare condition requiring a detailed expensive workup. Paraffin IF provides the correct diagnosis by unmasking immunoglobulins and light chains thus ruling out C3G in a significant minority of cases [39, 46]. Staining for tissue-bound C3d, or possibly C4d, in addition to the usual staining for soluble C3c might be useful to distinguish C3 glomerulopathies from immune complex glomerulonephritis, although C4d staining may also be present nonspecifically in normal glomeruli, making interpretation more challenging [57, 58, 65].

Membranous-like glomerulopathy with masked IgG kappa deposits is a specific lesion that requires paraffin IF for diagnosis [38, 41]. This is a disease predominantly of teen and young adult females (but with a wide age spectrum, 10–72 years), which presents with vague autoimmune symptoms. LM reveals segmental membranous glomerulopathy with corresponding segmental membranous-like subepithelial deposits by EM. Routine IF shows C3 only in mesangial and capillary loop regions with rare cases showing trace to 1+ IgG. Paraffin IF demonstrates characteristic IgG and kappa but not lambda staining. Subsequent confirmation can then be done using IHC for serum amyloid P protein [41].

Electron Microscopy

Tissue for EM is processed with post fixation in 1% osmium tetroxide, which enhances contrast of the tissue, and then dehydrated and embedded. With new, more rapidly polymerizing embedding media, such as Spurr, tissue may be ready for examination within 1 day. Ultrasound may further hasten the processing but may cause some artifacts. Electron microscopic study added information in a significant but small number of renal biopsies. In one study, EM was needed to make a diagnosis in 21% of cases and provided important confirmatory data in approximately 20% of cases [24]. EM showed diagnostic pathological abnormalities in 18% of patients with "normal" light microscopic findings. Larger, so-called thick scout sections (1 μ) made from the tissue allocated for EM are stained with toluidine blue to select smaller areas for thin sectioning for direct assessment with the electron microscope. Usually, the glomerulus containing the most representative lesion is chosen, but sclerotic glomeruli are not useful to examine by EM. If there are irregular or focal hypercellular/proliferative lesions, several glomeruli may be sampled, including the proliferative ones. The 60–90 Å thin sections are stained with uranyl acetate and lead citrate to enhance contrast before viewing in the EM scope.

Specific localization of immune complexes is done by EM examination, indicating whether deposits are subendothelial, subepithelial, mesangial, or in all of the above compartments. Immune complex deposits are nonmembrane-bound and denser than basement membrane or matrix materials. In specific diseases, such as cryoglobulinemia, amyloid, immunotactoid or fibrillary glomerulopathies, or lupus nephritis, specific substructure of deposits may be seen. In some diseases, such as light chain deposition disease or lupus nephritis, deposition may also be seen in vessels and tubules. So-called fingerprint deposits, with substructure reminiscent of fingerprinting, are present in some cases of lupus nephritis. Reticular aggregates (or so-called tubular arrays) of organelle membrane material in endothelial cell cytoplasm throughout the body are characteristically seen in large numbers in patients with SLE or HIV infection, or those treated with exogenous interferon, thought to reflect a response to high levels of interferon [61].

EM also delineates specific basement membrane abnormalities. For instance, small subepithelial deposits without surrounding new basement membrane material do not result in spikes and therefore cannot be visualized by Jones' stain on LM but can still be detected directly by EM. The lamina densa of the GBM is markedly thickened in diabetic nephropathy. Circumferential cellular interposition is defined by extension of monocytes and mesangial cell cytoplasm into the subendothelial space, with newly formed basement membrane interpositioning between the advancing infiltrating cells and the endothelium with intervening basement membrane material, thus giving rise to the classic double contours seen with silver stain by LM in MPGN. Increased lucent material is present in the expanded lamina rara interna in transplant glomerulopathy, HUS, eclampsia/preeclampsia, and other diseases presumed to involve endothelial injury/coagulopathy (see Chaps. "Renal Involvement in Children with Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP)," and ▶ 24, "Infection-Related Hemolytic Uremic Syndrome (HUS)"). In these conditions, deposition of fibrin, fibrinogen, and their degradation products and/or accumulation of platelets may also occur. Fibrin is recognizable by its dense, coarse sheaflike structure by EM, with periodicity observed in favorable sections. Morphometry of the GBM from EM images is used to diagnose thin basement membranes in hereditary nephritides (see ▶ Chap. 13, "Inherited Diseases of the Glomerular Basement Membrane"). EM also allows structural assessment of changes of specific cells. Diagnostic inclusions are seen in various storage/metabolic disease, such as Fabry's disease.

Normal Kidney Morphology

During fetal maturation, the glomerular capillary tufts are initially covered by large, cuboidal, darkly staining epithelial cells with only small lumina visible (Fig. 6). The cells lining Bowman's space undergo similar change from initial tall columnar to cuboidal to flattened epithelial cells, except for those located at the opening of the proximal tubule, where cells remain taller. Immature nephrons may occasionally be seen in the superficial cortex of children up to 1 year of age. Glomerular growth continues until adulthood, with average normal glomerular diameter approximately 95 μ in a group of patients less than 5 years old (average age 2.2 years) and 140–160 μ in adulthood [17, 30, 67].

The normal mature glomerulus consists of a complex branching network of capillaries originating at the afferent arteriole and draining into the efferent arteriole. The glomerulus contains three resident cell types: mesangial, endothelial, and epithelial cells (Figs. 7 and 8). The visceral epithelial cells (podocytes) cover the urinary surface of the GBM with pseudopodlike extensions called foot processes, with intervening filtration slits. Endothelial cells are opposed to the inner surface of the GBM and are fenestrated. At the stalk of the capillary, the endothelial cell is

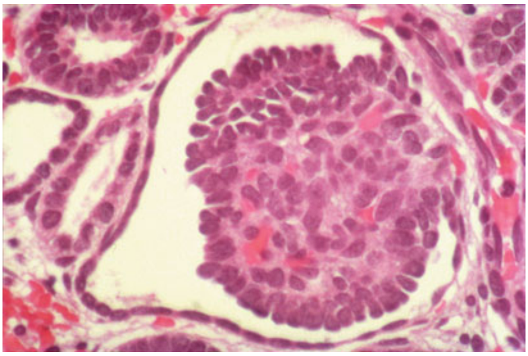


Fig. 6 Immature glomerulus with plump, dark epithelial cells from biopsy of a 29-week-gestation baby (PAS, original magnification ×670)

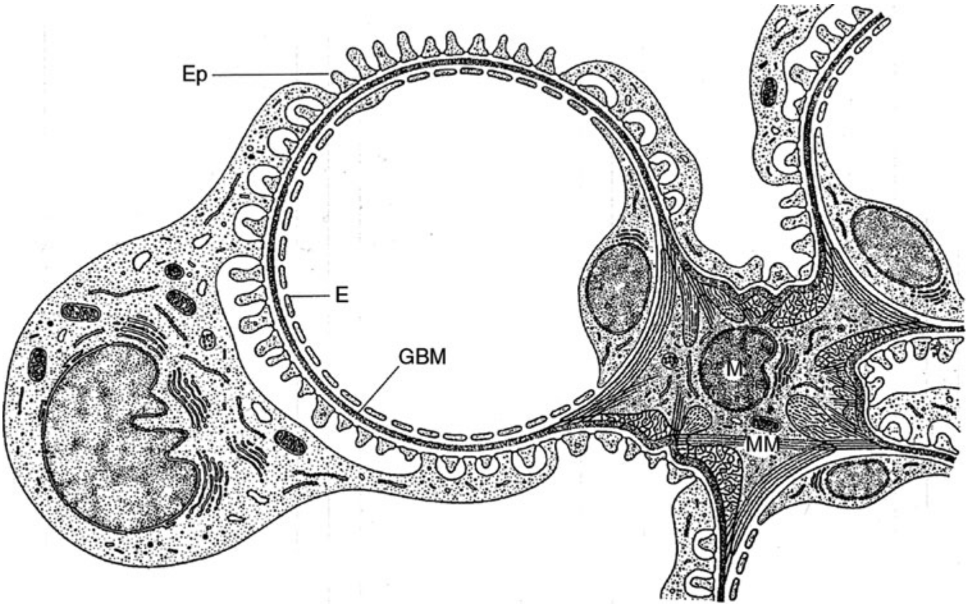


Fig. 7 Schematic illustration of glomerulus, with glomerular capillary attached to a mesangial stalk area. The glomerular endothelium (*E*) is fenestrated and lines the glomerular basement membrane (*GBM*), which covers the mesangium. The outside of the GBM is covered by the epithelial cell and its foot processes (*Ep*). The mesangial cell (*M*) is embedded within the mesangial matrix (*MM*), with processes connecting to the GBM (Provided courtesy of Professor Wilhelm Kriz)

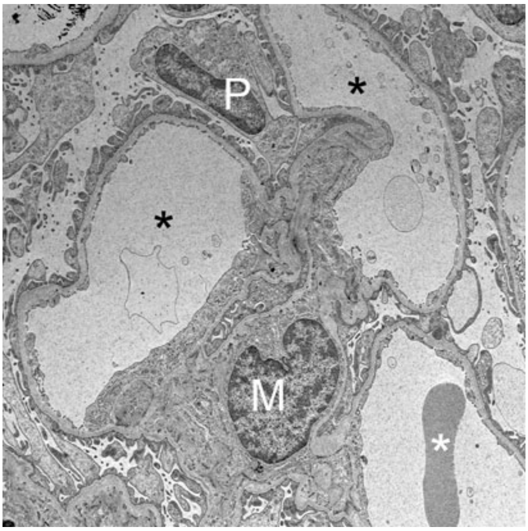


Fig. 8 A normal glomerulus examined by transmission electron microscopy. There are three capillary loops with the capillary lumina marked by black *, with red blood cells marked by a white *. A podocyte nucleus on the outside of the glomerular basement membrane with interdigitating foot processes is marked with a P. The inside of the glomerular basement membrane is lined with fenestrated endothelium. A mesangial cell (M) lodges in the mesangial stalk, anchoring the capillary loops (transmission electron microscopy, original magnification ×2000)

separated from the mesangial cells by intervening mesangial matrix. The term *endocapillary* is used to describe hypercellularity/proliferation filling up the capillary lumen, contributed to by proliferation of mesangial, endothelial, and infiltrating inflammatory cells. In contrast, *extracapillary* hypercellularity/proliferation refers to an increased number of parietal epithelial cells that line Bowman's capsule, resulting in a crescent lesion.

The mesangial cell is a contractile cell that also has phagocytic properties. It lies embedded in the mesangial matrix in the stalk region of the capillary loops, attached to anchor sites at the ends of the loop by thin extensions of its cytoplasm. Normally up to three mesangial cell nuclei per lobule are present. The basement membrane consists of three layers distinct by EM, the central broadest lamina densa and the less electron-dense zones of lamina rara externa and interna. Thickening occurs with maturational growth. Most investigators have found thicker basement membranes in boys, with normal range from 220 to 260 nm at 1 year of age, 280 to 327 nm at age 5 years, 329 to 370 nm at age 10 years, and 358 to 399 nm at age 15 years [48]. In our laboratory, we found a range

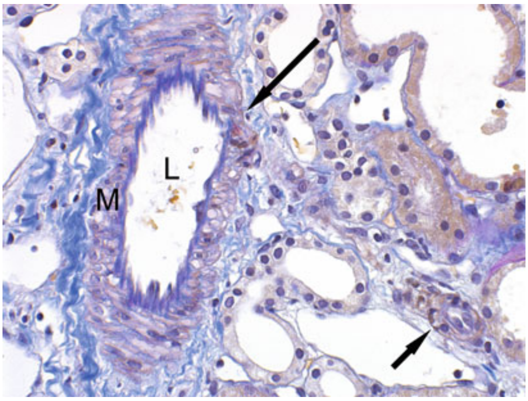


Fig. 9 A normal artery is shown on the left, marked by the long arrow, with the lumen (L) and the media (M). Arteries are distinguished from arterioles (short arrow) by having at least two layers of smooth muscle cells in the media (trichrome-elastica, original magnification ×400)

of GBM thickness in children with normal kidneys from approximately 110 nm at age 1 year to 222 ± 14 nm at 7 years of age.

The glomerulus is surrounded by Bowman's capsule, which is lined by parietal epithelial cells. These are continuous with the proximal tubule. The efferent and afferent arterioles can be distinguished morphologically in favorably oriented sections or by tracing their origins on serial sections. Segmental, interlobular, and arcuate arteries may also be present in the renal biopsy specimen (Fig. 9). The cortical biopsy also allows assessment of the tubules and interstitium. Proximal tubules are readily identified by their PAS-positive brush border, lacking in the distal tubules. Collecting ducts show cuboidal, cobblestone-like epithelium. Tubules are normally back-to-back with minimal interstitial cells and the peritubular capillaries intervening (Fig. 10a). The medulla may also be included in the biopsy.

Patterns of Injury

Overall Pattern

Assessment of the biopsy specimen must include inspection of all sections from different levels because additional glomeruli may be sampled on deeper cuts of the biopsy core and many diseases are characterized by focal lesions [9]. The severity and patterns of lesions are assessed, and normal and affected glomeruli are counted. Lesions are classified as focal if only some (or less than half) glomeruli are involved, diffuse if all (or most) glomeruli are involved, segmental if only portions of glomeruli are involved, and global if entire glomerular tufts are involved. Characteristic glomerular disease patterns include lobular hypercellularity with double contours of the GBM in MPGN, nodular sclerosis with increased mesangial cells, and abundant matrix material in characteristic Kimmelstiel-Wilson lesions in diabetic nephropathy, focal and segmental sclerosis, intraglomerular/arteriolar fibrin thrombi, necrotizing lesions, and crescent formation (Table 1) [25]. The crescent, a lesion due to increase of mostly parietal epithelial cells, owes its name to its shape in well-established lesions.

Glomeruli are assessed for alterations in size (see below). It is important to compare with a normal control for a given age group because marked glomerular maturational growth occurs in children. Glomerular hypertrophy may be an important predictor of increased risk for ultimate development of FSGS in children with apparent minimal change disease (MCD) [17] (see ▶ Chaps. 14, "Steroid-Sensitive Nephrotic Syndrome," and ▶ 15, "Non-genetic Forms of Steroid-Resistant Nephrotic Syndrome"). Maturational pattern of glomeruli (see above) should be noted. Occasional fetal glomeruli may be found in children beyond infancy and are not of specific diagnostic significance.

Glomeruli are assessed for glomerulosclerosis, that is, the presence of segmental obliteration and scarring of glomerular capillary tufts. Sclerosis may be in a segmental or global pattern (Table 1). Previous studies suggested that up to 10% of glomeruli may be normally globally sclerosed in people younger than 40 years of age [33]. This number may be even smaller in children, with less than 1–3% global sclerosis expected normally up to age 40 or 56, respectively [34, 64]. These occasional globally sclerotic glomeruli are thought to represent errors of nephrogenesis. The percentage of global sclerosis increases even with normal aging, up to half the patient's age, minus 10 [64]. Globally sclerosed glomeruli in greater percentage indicate the possibility of renal disease (focal global sclerosis).

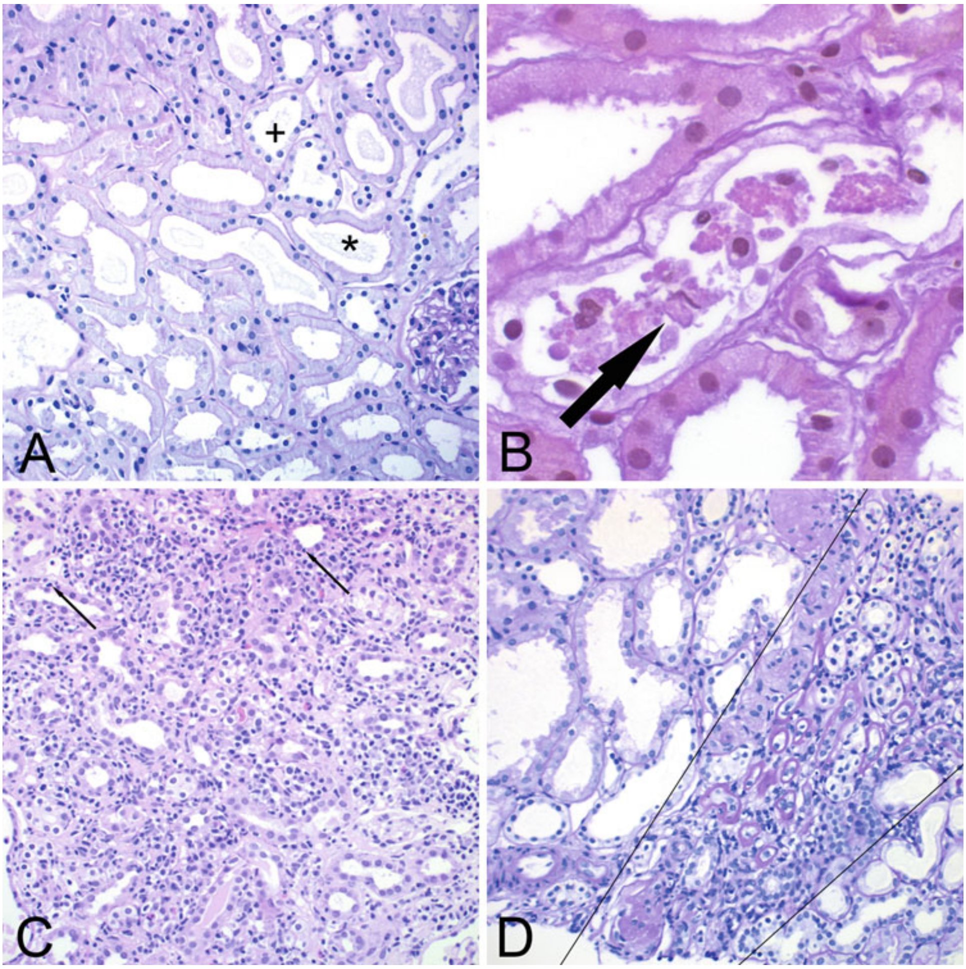


Fig. 10 Normal tubules and tubules in various injuries. (**a**) Normal cortical tubules with proximal segments (*) showing a well-developed periodic acid-Schiff-positive brush border and a distal segment (marked with +) without brush border (PAS, original magnification ×200). (**b**) Acute tubular damage with cellular debris in the lumen (arrow) and flattened tubular epithelium with attenuated brush border (trichrome, original magnification x630). (**c**) Acute tubulointerstitial nephritis with edema and infiltrate of lymphocytes, histiocytes, plasma cells, and eosinophils surrounding and infiltrating tubules (tubulitis) with acute tubular injury (arrows) (H&E, original magnification ×200). (**d**) Focal cortical tubular atrophy and interstitial fibrosis outlined by two black lines. These atrophic tubules have much smaller diameter compared to the surrounding tubules and thicker basement membranes and are surrounded by fibrotic, expanded interstitium with mononuclear infiltrates (PAS, original magnification ×200)

Selected Specific Patterns of Injury

Mesangial Hypercellularity

Hyperplasia of mesangial cells is defined as more than three mesangial cell nuclei present per mesangial region, in areas away from the vascular pole (Fig. 11). Increased mesangial prominence may be due to increased cellularity, increased matrix, deposits, or a combination. Large mesangial deposits appear on Jones' stain as pinkish areas surrounded by the light silver-staining areas of mesangial matrix. So-called interposition results when monocyte or mesangial cell cytoplasm extends outward between basement membrane and endothelial cells and new matrix accumulates between the mesangial and endothelial cell bodies.

Table 1 Definitions of common morphological terms (Modified from [25])

Light microscopy	
Focal	Involving some glomeruli (some definitions use this term for lesions involving <50% of, e.g., glomeruli)
Diffuse	Involving all glomeruli (some definitions use this term for lesions involving ≥50% of, e.g., glomeruli)
Segmental	Involving part of glomerular tuft (some definitions use this term for lesions involving <50% of the glomerular tuft)
Global	Involving total glomerular tuft (some definitions use this term for lesions involving ≥50% of the glomerular tuft)
Lobular	Simplified, lobular appearance of capillary loop architecture (MPGN)
Nodular	Rounded accumulation of collagenous matrix expanding one or more mesangial areas (e.g., in diabetic nephropathy)
Sclerosis	Obliteration of capillary loop by increased matrix (scarring)
Crescent	Extracapillary hypercellularity composed of mostly parietal epithelial cells, involving >10% of the capsular circumference, more than two cell layers thick
Spikes	Projections of glomerular basement membrane intervening between subepithelial immune deposits (e.g., in membranous GN)
Endocapillary proliferation/ hypercellularity	Increased cells within capillary lumens causing luminal narrowing or occlusion
Hyaline	Descriptive of glassy, smooth-appearing material
Hyalinosis	Hyaline-appearing (glassy, smooth) PAS positive, silver negative, amorphous, eosinophilic insudation, composed mainly of plasma components (in, e.g., glomeruli in focal segmental glomerulosclerosis or in arterioles in, e.g., hypertension or diabetes)
Mesangium	Stalk region of capillary loop with mesangial cells surrounded by matrix
Subepithelial	Between podocyte and glomerular basement membrane
Subendothelial	Between endothelial cell and glomerular basement membrane
Tram-track	Two or more contours of glomerular basement membrane due to deposits and/or interposition of cells (see below)
Wire loop	Thick, rigid appearance of capillary loop due to large subendothelial deposits, seen by LM
Activity	Extent of possible treatment sensitive lesions, based on, e.g., extent of cellular/ fibrocellular crescents, cellular infiltrate, necrosis, proliferation
Chronicity	Extent of probable irreversible lesions, based on, e.g., extent of tubular atrophy, interstitial fibrosis, fibrous crescents, glomerulosclerosis
Immunofluorescence microscopy	
Granular	Discontinuous flecks of staining along capillary loop producing granular pattern
Linear	Smooth continuous staining along capillary loop
Electron microscopy	
Foot process effacement	Flattening of foot processes of podocytes so that they cover the basement membrane with loss of slit diaphragm junctions
Microvillous transformation	Small extensions of podocytes with villus-like appearance
Interposition	Extension of cell cytoplasm with interposition between endothelial cell cytoplasm and basement membrane and underlying new basement membrane formation
Reticular aggregates	Organized arrays of interlacing, curved tubular structures within the endoplasmic reticulum cisternae in endothelial cells
Immunotactoid GP	Large, organized microtubular deposits, >30 nm diameter
Fibrillary GP	Fibrils 14–20 nm diameter without organization (usually DNAJB9 positive by IHC)

GP glomerulopathy, *IHC* immunohistochemistry, *MPGN* membranoproliferative glomerulonephritis

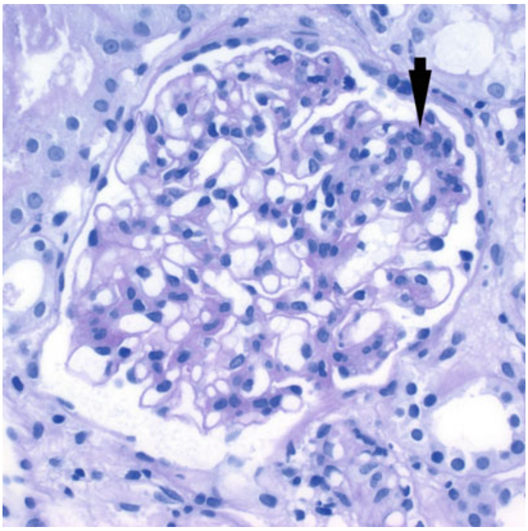


Fig. 11 Segmental mesangial hypercellularity (arrow), defined as a cluster of more than 3 mesangial cells, recognized by being surrounded by mesangial matrix, in a mesangial area (PAS, original magnification ×400)

Endocapillary Hypercellularity/Proliferative Lesions

Endocapillary cell hypercellularity/proliferation (defined as increased number of cells within the capillary lumen, causing significant narrowing of the capillary or occlusion) is characteristic of for example diffuse proliferative lupus nephritis and the MPGN pattern (Fig. 12). This pattern most often is a response to subendothelial deposits. The specific diagnosis requires IF and EM studies and integrated clinicopathologic interpretation.

Membranoproliferative Pattern

The glomeruli show a lobular appearance, with mesangial and often endocapillary hypercellularity (see above) and double contours of the GBM (Fig. 13). This is often caused by subendothelial deposits with interposed cells reacting to the deposits but may also result from chronic endothelial injury with increased lamina rara interna and interposed cells without deposits (i.e., chronic TMA, or more correctly, chronic microangiopathy without thrombosis). The deposits may be due to immune complexes, C3 glomerulopathy, monoclonal protein, or other entities. Thus, IF and EM are crucial to distinguish the cause of MPGN pattern lesions.

Exudative Pattern

This describes a hypercellular glomerulus with prominent inflammatory cells including frequent PMNs narrowing or occluding capillary lumens and is most often seen in infection-related immune complex GN (Fig. 14).

Crescents

Crescents consist primarily of proliferating parietal epithelial cells with some infiltrating macrophages and are a manifestation of severe glomerular injury (Fig. 15). The name reflects the often crescent-shaped sheet of cells filling up part or nearly all of Bowman’s space. Crescents result from injuries that break the GBM, leading to exudation of plasma protein and formation of fibrin within Bowman’s space, which then induces proliferation of the parietal epithelial cells and infiltration of macrophages. When crescents are a prominent histologic feature, the patient most often presents clinically with a rapidly progressive glomerulonephritis. Crescents may occur in a variety of diseases. Diseases with crescents as a primary manifestation include antibody-mediated injury (anti-GBM antibody disease), severe immune-complex diseases (e.g., lupus nephritis), and pauciimmune diseases, the latter most often ANCA positive. The early lesion of cellular crescents is responsive to cytotoxic therapy. Biopsy indications of irreversible renal damage include breaks of Bowman’s capsule [10] and fibrous transformation of the cellular crescents, periglomerular fibrosis, and scarred glomeruli and tubulointerstitium.

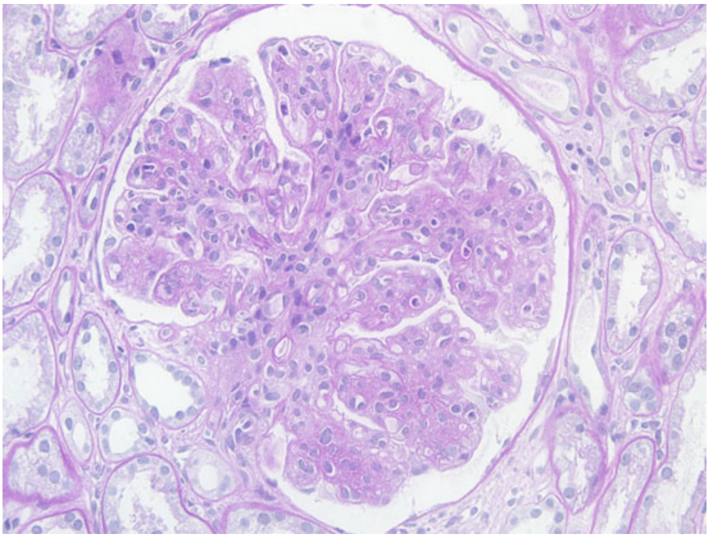


Fig. 12 Global endocapillary hypercellularity which is defined as increased number of cells within capillary lumens causing luminal narrowing or occlusion (PAS, original magnification ×400)

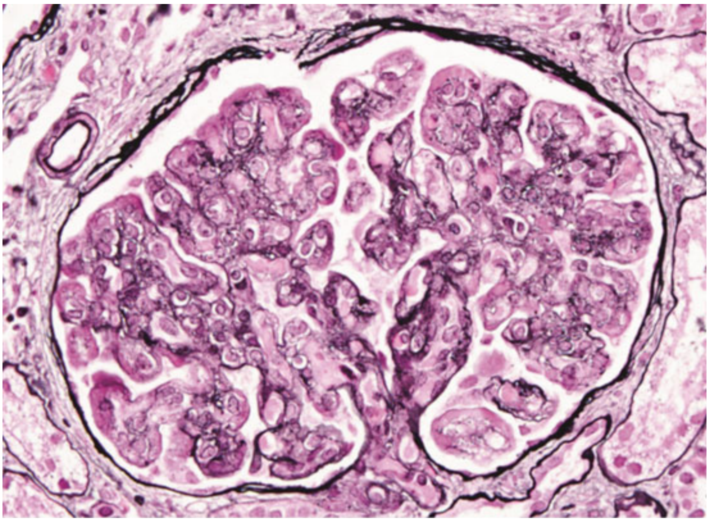


Fig. 13 Double contours of the glomerular basement membrane ("tram-tracking") in membranoproliferative glomerulonephritis (MPGN), caused by subendothelial/intramembranous deposits and cellular interposition (Jones' silver stain, original magnification ×400)

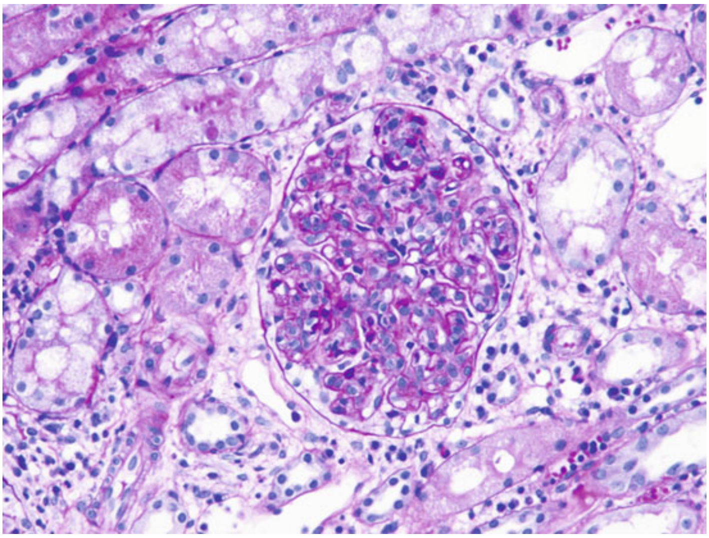
Segmental Sclerosis

Segmental sclerosis refers to sclerosis of a portion but not the whole glomerulus (Fig. 16). In contrast, global sclerosis refers to sclerosis of the entire tuft, and when present in an obsolescent pattern is nonspecific and can occur at any age, even in normal young children. Segmental sclerosis of classic type is defined as obliteration of the capillary lumen by increased matrix. There may be associated hyalinosis, defined as insudation of components of plasma, including lipids, which stain PAS positive and appears glassy smooth (hence the term hyaline. Foam cells may be present. Segmental sclerosis can happen due to any chronic glomerular injury and specific diagnosis must be made with integration of sclerosis type, immunofluorescence, and electron microscopy findings and clinical setting (see sections on specific diseases).

Thrombosis

Thrombosis may involve glomeruli or the vasculature, including arterioles and arteries, and is

Fig. 14 Postinfectious glomerulonephritis with endocapillary hypercellularity/proliferation and neutrophil infiltration (PAS, original magnification ×430)



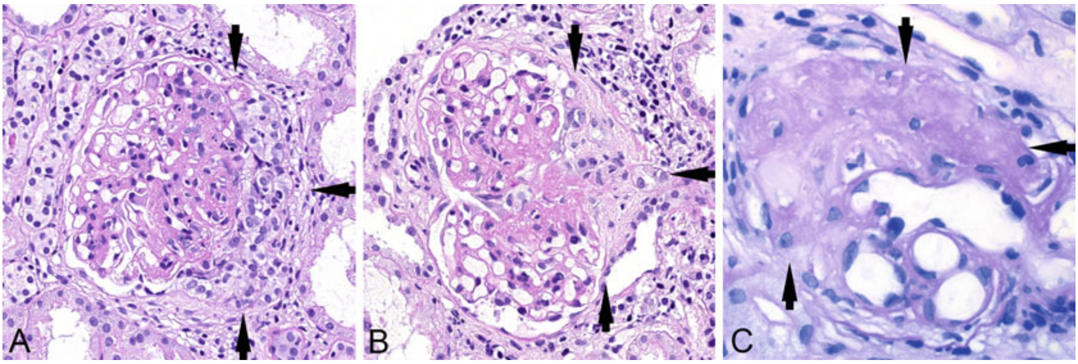


Fig. 15 Stages of crescents, defined by extracapillary hypercellularity surrounding the glomerular tuft (**a**) A cellular crescent (arrow), an active lesion, defined as consisting of more than two cell layers, with more than 75% of the crescent composed of cells (PAS, original magnification ×400). (**b**) Fibrocellular crescent (arrows), the intermediate stage of organization and scarring, with 25–75% of the crescent composed of cells, and the remainder of matrix (PAS, original magnification ×400). (**c**) Fibrous crescent, the most advanced stage, composed of at least 75% matrix, with correspondingly decreased cellular content. Note the four remaining capillary lumina (PAS, original magnification ×630)

defined as intralumenal fibrin thrombi composed of fibrin, platelets, and coagulated blood (Fig. 17). The term thrombotic microangiopathy has also been used to describe associated vascular lesions with fibrinoid necrosis within the wall, but not in the lumen, or chronic microangiopathy changes without fibrin, better called microangiopathy without thrombosis (see ▶ Chaps. 21, "Autoantibodies and Kidney Diseases," "Renal Involvement in Children with Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP)," and ▶ 24, "Infection-Related Hemolytic Uremic Syndrome (HUS)") [22].

Acute Tubular Injury

Morphologically evident acute tubular injury or necrosis correlates poorly with the clinical extent of acute kidney injury (AKI) (Fig. 10b). The changes vary from nondiagnostic vacuolization to frank necrosis with sloughing of tubular epithelial cells (toxic type) and flattened epithelium

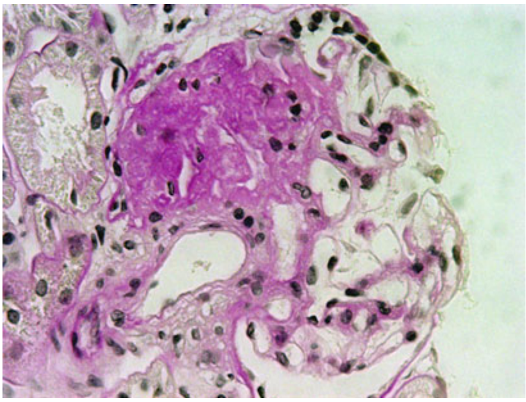


Fig. 16 Segmental sclerosis, with a portion of the tuft showing obliteration of the capillary lumens by matrix (PAS, original magnification ×400)

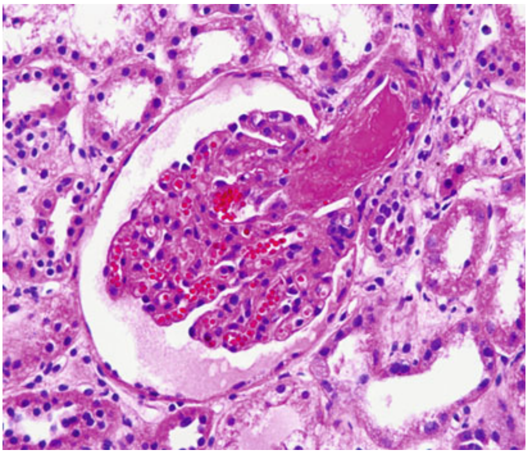


Fig. 17 Arteriolar fibrin thrombi with congestion in capillary loops, characteristic of the thrombotic microangiopathy seen in hemolytic uremic syndrome (Jones' stain, original magnification ×270)

characteristic of regeneration (ischemic type). In cortical necrosis, zones of cortex, including glomeruli, are necrotic.

Interstitial Fibrosis/Tubular Atrophy (IFTA)

In chronic kidney disease, tubules are atrophied with dilation and flattened epithelium (Fig. 10d). IFTA may occur secondary to lesions affecting the glomerulus, although primary tubular and interstitial injury mechanisms may also cause IFTA. Increased IFTA is associated with worse prognosis regardless of its cause [55]. Fibrosis results in increased spacing of tubules because of the accumulation of PAS-positive collagenous material, with thickened tubular basement membranes. The collagen also stains specifically blue with Masson's trichrome stain. Fibrosis in a striped pattern suggests calcineurin inhibitor toxicity or other causes of ischemia (e.g., renal artery stenosis), with the striped pattern reflecting particular susceptibility of the medullary rays to such hypoxic injury (see ▶ Chap. 81, "Renal Transplantation: Immunosuppression and Managing Allograft Rejection"). Acute tubular injury may result in regeneration, or if cell cycle arrest occurs, develop into IFTA lesions.

Acute Interstitial Nephritis

Interstitial edema is a nonspecific change, present, for example, in early acute transplant rejection, renal vein thrombosis, or inflammatory processes. Acute interstitial nephritis (AIN) is defined as edema and lymphoplasmacytic infiltrate, with variable components of other inflammatory cells (Fig. 10c). Eosinophils in an infiltrate are suggestive of drug-induced/hypersensitivity interstitial nephritis, although eosinophils are also present in some cases of idiopathic interstitial nephritis and in diabetic nephropathy with IFTA [54]. Of note, the lack of eosinophils does not exclude hypersensitivity as the etiology of the AIN. Eosinophils can also be part of acute cellular rejection in the transplant. Nonnecrotizing granulomas, with or without eosinophils, most often reflect drug-induced hypersensitivity reaction. A pleomorphic infiltrate of lymphocytes, plasma cells, and PMNs suggests possible polyoma virus nephropathy in the transplant, confirmed by finding viral nuclear inclusions and positive immunostaining in tubules. Polyoma virus can also cause tubulocentric granulomas in some cases. Plasma cell-rich infiltrates with a whorling storiform fibrosis pattern suggest IgG4-associated interstitial nephritis.

Prognostic Implications of Biopsy Findings

When the biopsy sample is adequate, extensive, severe, and irreversible lesions signify a dismal prognosis for the patient. Globally sclerotic glomeruli are not amenable to treatment, although evidence from human diabetic nephropathy and animal studies indicates that the earlier stages of sclerosis may be affected by some therapeutic interventions and may even be reversible. Similarly, active lesions with ongoing cellular crescents, necrosis, and inflammatory infiltrates are potentially dramatically modulated by therapy, allowing subsequent healing. There may be minimal irreversible damage to glomerular structures when intervention occurs early.

Although the renal biopsy may yield a diagnosis, there is less information about prognostic indicators in diseases that have a variable course. Extensive analysis aimed at determining histologic features associated with poor prognosis has been done in some diseases, discussed in specific sections. The best prognostic indicator in one study of lupus nephritis was the proportion remaining of intact glomeruli in follow-up biopsies [28].

New Methods for the Future

With the recent surge of the application of molecular biology techniques to the study of renal disease, candidate factors involved in pathogenesis and progression of disease are being studied in animal models. Studies in human beings have also commenced. With further development of such studies, we may identify specific abnormal processes and thus target therapy more specifically. Research techniques that have been advantageously applied to elucidate pathogenesis of disease include immunostaining; identifying the specific antigen in deposits of membranous glomerulopathy; light chains or paraproteins in plasma cell dyscrasia-associated diseases; identification of the specific type IV collagen abnormality in Alport syndrome; C4d as a marker of antibody-mediated rejection, novel insights into C3 glomerulopathies and elucidation of pathogenesis of specific *E. coli*-associated toxins in some forms of HUS.

Current studies are aimed at understanding disease etiologies and mechanisms at a molecular and proteomic level, studying renal biopsies by laser capture microdissection (LCM), with mass spectrometry, real time reverse transcription polymerase chain reaction (RT-PCR), and in situ hybridization techniques including nanostring technology [19, 45, 56, 59, 75]. Recent efforts have expanded use of these techniques to study mechanisms of injury and progression. Assessment of the so-called “Increased Expression of Thoroughly Validated Gene Transcript/Classifiers in the Biopsy Tissue Strongly Associated with AMR,” although not officially endorsed, may help in the diagnosis of antibody-mediated rejection [52]. Modulation of growth factors, collagens, cytokines, chemokines, and their receptors has been investigated molecularly in renal biopsies. Single cell RNA seq from kidney tissue and even urine is elucidating new cell types of the normal kidney and novel pathways in disease [2, 76]. Such approaches can offer exciting new mechanistic insights into renal diseases.

In parallel, genetic studies are targeted both at diagnosis and identifying patients at risk for progression in diseases with a variable course. Genetic screening for key podocyte-related mutations is often done in children with FSGS. Autosomal dominant tubulointerstitial diseases often do not show distinct pathologic features, and genetic studies are necessary for precise diagnosis. Noninvasive urinary proteomic and microvesicle studies are also being explored to identify particular signature patterns indicative or predictive of specific lesions or risks [2].

Together these approaches promise to map risks and mechanisms of disease initiation and progression and point to targets to achieve resolution of injury. Sequential biopsies with evaluation of changes in structure and patterns of abnormal factors and modulation by therapy may be necessary to fully understand pathogenesis. Instead of diagnoses of morphologic patterns recognized by current techniques in the renal biopsy specimen, molecular techniques may allow more precise diagnosis of the specific diseases and identification of injury mechanisms.

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Approach to the Child with Hematuria and/or Proteinuria

8

Hui-Kim Yap and Mohan Shenoy

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Abstract

Hematuria and proteinuria are among the common reason why children are referred to a pediatric nephrologist. The initial step in a child with hematuria is to establish the diagnosis by performing a urine microscopy to look for the presence of >5 RBC/microliter in a fresh uncentrifuged urine specimen. The prevalence of isolated hematuria is reported to be 0.4–0.5% in large epidemiological studies. There is increasing evidence that persistent hematuria, particularly if associated with proteinuria, is a risk factor fo chronic kidney disease in the longer term. Therefore, careful history and clinical examination is important so that appropriate investigations are undertaken to identify the

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etiology. Glomerular causes of hematuria (glomerulonephritis and familial hematuria) are more common in children than lower tract causes of hematuria (cystitis and hypercalciuria). There is increasing role for genetic testing in children with hematuria, particularly where there is family history of hematuria, deafness, or chronic kidney disease. Imaging (ultrasound and CT scan) is important if urolithiasis or a structural abnormality is suspected.

Proteinuria can be nonpathological (febrile, exercise, or orthostatic) or pathological. Children with persistent proteinuria can be asymptomatic or they can present with nephrotic syndrome. Spot first morning urine sample for urine protein: creatinine ratio (PCR), or albumin: creatinine ratio (ACR) is the ideal test to confirm the presence of proteinuria. Children with urine PCR >50 mg/mmol (>1 g/1.73m^2/day) merit further evaluation in order to exclude significant pathology like primary or secondary glomerulonephritis. Renal biopsy should be considered in children with persistent proteinuria, especially if it is associated with hematuria, reduced GFR, or hypertension.

Keywords

Hematuria · Proteinuria · Glomerulonephritis · Familial nephritis · Kidney biopsy

Synonyms

Autosomal alport syndrome; Henoch-schonlein purpura; IgA vasculitis; TBMN

Approach to the Child with Hematuria

Introduction

Hematuria is a common finding in children. It is defined as the presence of increased red blood cells (RBC) in the urine. The definition varies according to the mode of detection, that is, greater than 5 RBC/microliter in a fresh uncentrifuged midstream urine specimen and greater than 3 RBC/high power field in a centrifuged sediment from 10 ml of freshly voided midstream urine.

Hematuria may either be macroscopic or microscopic. Macroscopic hematuria may appear as red, smoky dark brown, or cola-colored urine. Other causes of pigments in the urine must be considered as differential diagnoses. Dark brown or black urine could be due to bile pigments, methemoglobinemia, alanine, cascara from the dried skins of the coffee fruit, drugs such as resorcinol and thymol, alkaptonuria, and tyrosinosis. Red or pink urine could be due to free hemoglobin, myoglobin, porphyrins, high concentration of urates, foods such as beetroot, blackberries, dragon fruit and red dyes, and drugs including benzene, chloroquine, desferoxamine, phenazopyridine, and phenolphthalein. Microscopic hematuria on the other hand is usually first suggested by the presence of "blood" on a urine dipstick examination. Oxidation of the chromogen tetramethylbenzidine on the dipstick by hemoglobin peroxidase in the presence of hemoglobin or myoglobin results in a green-blue color. This test is very sensitive and can detect as little as 150 microgram/liter of free hemoglobin. Unfortunately, false positive results are common, and is usually due to oxidizing agents present in the urine in particular microbial peroxidases associated with urinary tract infection.

Epidemiology

Epidemiological studies on the prevalence of hematuria in school-aged children indicate that 0.5 to 1% of them will have hematuria detected on two or more occasions, with at least one-third persistent beyond 6 months [1–5]. Large-scale screening programs primarily from Asia involving 350,000 school children from Japan [4] and 40,000 from China [6] reported isolated hematuria in 0.4–0.52%, while 0.03–0.7% of children had both hematuria and proteinuria. In another study involving 1 million young persons aged 16 through 25 years, undergoing medical examination for military service in Israel, persistent isolated microhematuria was found in 0.3%, with males being affected twice as commonly as females [7].

There is increasing evidence from epidemiological studies that hematuria is a risk factor for progression of chronic kidney disease [8, 9]. Evaluation of persistent hematuria with or without proteinuria by renal biopsy identified glomerulonephritis as a cause in 25.4–52.3% [4, 10, 11]. Adverse kidney events, defined by development of proteinuria, hypertension, or impaired kidney function, were observed over a two- to ten-year follow-up period in 6% of children with asymptomatic isolated microscopic hematuria, while 28% of those with hematuria and proteinuria had evidence of progressive kidney disease [11]. Data from the large population-based registry in Israel showed an increased risk of end-stage kidney disease attributable to glomerular disease in adolescents and young adults with isolated microhematuria compared to those without, with incidence rates of 19.6 versus 0.55 per 100,000 patient-years, respectively [7]. The fraction of treated end-stage kidney disease attributed to microscopic hematuria was 4.3% (95% CI, 2.9–6.4%) in that study.

Etiology

Hematuria may originate from the glomeruli, renal tubules and interstitium, or urinary tract (including collecting systems, ureters, bladder, and urethra). In children, glomerular causes of hematuria occur more commonly than non-glomerular. Table 1 outlines the various causes of hematuria. Hematuria can be macroscopic or microscopic.

Table 1 Causes of hematuria classified as of glomerular or non-glomerular origin

Glomerular Hematuria
Familial hematuria syndromes
Thin basement membrane nephropathy
Alport syndrome
Hereditary angiopathy, nephropathy, aneurysms, muscle cramps syndrome
MYH9 syndromes (associated thrombocytopathies)
C3 glomerulopathy
Glomerulonephritis
Postinfectious glomerulonephritis
Membranoproliferative glomerulonephritis
Membranous nephropathy
Crescentic glomerulonephritis
IgA nephropathy
IgA vasculitis nephritis (Henoch-Schonlein nephritis)
Lupus nephritis
Necrotizing vasculitis
Hemolytic uremic syndrome
Non-glomerular Hematuria
Urinary tract infection (bacterial, viral and parasitic)
Hypercalciuria
Urolithiasis
Trauma
Exercise-induced
Chemical cystitis
Cystic kidney disease
Vascular malformations
Nutcracker syndrome
Papillary necrosis
Renal infarction
Malignancies
Nephroblastoma (Wilms tumor)
Renal cell carcinoma
Rhabdomyosarcoma of the bladder
Bleeding disorders
Renal vein thrombosis
Menarche
Factitious

Lower urinary tract causes of gross hematuria include hemorrhagic cystitis (infection or drug induced), urethritis or perineal irritation, urolithiasis, trauma, exercise, coagulopathies, and rarely bladder malignancies [12]. In certain communities in Africa and Asia, *Schistosomia hematobium* is an important cause of hematuria and proteinuria which may lead to lower urinary tract dysfunction and chronic kidney disease [13]. Exercise-induced hematuria is transient occurring after severe exercise and resolving within 48 h. Bleeding disorders such as hemophilia or thrombocytopenia can result in both microscopic and gross hematuria. Renal infarction presenting with gross hematuria can occur in children with sickle cell disease [14].

Of the nonglomerular causes, hypercalciuria is seen in 26–36% of children with gross or microscopic hematuria [15–17]. Hypercalciuria is associated with a strong family history of urolithiasis [18, 19], with a suggestion that this may have an autosomal dominant inheritance pattern [20]. The nutcracker syndrome is another cause of nonglomerular hematuria [21, 22] that appears more commonly in Asian communities. Hematuria occurs following compression of the left renal vein between the superior mesenteric artery and the aorta. In Japan and Korea, the ultrasonographic findings consistent with the nutcracker syndrome have been described in 30–45% of children with hematuria [23, 24]. These children are usually asthenic in habitus, and present with either microscopic or macroscopic hematuria, which may be associated with left flank pain and left varicocele in boys. Symptoms resolve in up to 75% of children over 24 months, following weight gain which is associated with an increase in retroperitoneal adipose tissue deposition resulting in a decrease in left renal vein hypertension [25, 26].

On the other hand, macroscopic hematuria which is glomerular in origin usually presents as dark red or cola-colored urine, often occurring together with upper respiratory tract symptoms (synpharyngitic). The child may have recurrent episodes of gross hematuria, and this is seen with many of the glomerulonephritis and familial hematuria syndromes that are progressive, in particular Alport syndrome. Postinfectious glomerulonephritis is still the most common etiology in children presenting with the full-blown symptoms of acute nephritis, that is, cola-colored urine, edema, and hypertension. However, the other systemic causes of glomerulonephritis in children must be considered, in particular IgA vasculitis (Henoch-Schönlein nephritis) and lupus nephritis.

Microscopic hematuria is often asymptomatic, with the most common causes being glomerulopathies in particular postinfectious glomerulonephritis, immunoglobulin A (IgA) nephropathy, and the familial hematuria syndromes as outlined in Table 2 [27]. Of the familial hematuria syndromes involving mutations in the α-chains of the collagen IV gene (*COL4A1* to *COL4A6*) (Table 2) [28], thin basement membrane nephropathy (TBMN), also known as benign familial hematuria, is the most common cause of persistent microscopic hematuria. It is now increasingly recognized that TBMN and Alport syndrome have a common molecular basis [29]. Alport syndrome (AS) is a progressive hematuric glomerulopathy associated with ultrastructural changes of the glomerular basement membrane, sensorineural deafness, and ocular abnormalities [29–33]. The X-linked form due to a *COL4A5* gene mutation is the most common form occurring in 85% of patients. Autosomal Alport syndrome due to biallelic (homozygous or compound heterozygous) mutations in *COL4A3* or *COL4A4* genes behaves in an autosomal recessive manner, and is associated with a 100% risk of progression to end-stage kidney disease, the timing of which is dependent on the genotype. Heterozygous mutations in *COL4A3* or *COL4A4* may exhibit a dominant form of inheritance and include those who have been diagnosed with TBMN. It is now recognized that TBMN is a spectrum of autosomal Alport syndrome, where the risk of progressive glomerulopathy is up to 20% in the presence of factors such as proteinuria, sensorineural deafness, family history of end-stage kidney disease and histological findings of focal segmental glomerulosclerosis, or glomerular basement membrane thickening and lamellation. Digenic Alport syndrome includes patients with mutations in two of the *COL4A3*, *COL4A4*, or *COL4A5* genes. Those with *COL4A3* and *COL4A4* mutations in the trans position on the alleles exhibit an autosomal recessive inheritance pattern, 100% of whom will have progressive glomerulopathy, whereas the same two mutations in the cis position exhibit an autosomal dominant inheritance pattern with up to 20% showing progression. Males with *COL4A5* and either *COL4A3* or *COL4A4* all progress to end-stage kidney failure.

Other genetic forms of glomerular hematuria include IgA nephropathy which is one of the most common glomerulopathies worldwide. In countries where IgA nephropathy is prevalent, 10 to 35% of children undergoing renal biopsy for isolated hematuria were found to have the disease [34, 35]. Children with IgA nephropathy present commonly either with recurrent gross hematuria associated with upper respiratory tract infections or with asymptomatic hematuria with or without proteinuria. Familial occurrence has been described in this genetically complex multifactorial disease [36, 37].

Another cause of familial hematuria is seen in the group of disorders known as C3

Table 2 Familial hematuria syndromes

Inheritance	Gene(s)	Protein	Estimated risk of ESKD* (Median age)
X-linked Alport syndrome	*COL4A5* Hemizygous (male) Homozygous (female)	α5(IV)	100% (25 years) Up to 25% (49 years)
Autosomal Alport syndrome	*COL4A3* or *COL4A4* Recessive (homozygous or compound heterozygous) Dominant	α3(IV) or α4(IV)	100% (15 years) $<1\%$ if no risk factors (TBMN**); $\geq 20\%$ if risk factors present (60 years)
Digenic Alport syndrome	*COL4A3* and *COL4A4* Mutations in trans Mutations in cis Mutations in *COL4A5* and in *COL4A3* or *COL4A4*	α3(IV) and α4(IV)	Up to 100% Up to 20% Up to 100% (affected males)
Autosomal dominant macrothrombocytopathies	*MYH9* Heterozygous	Nonmuscle myosin heavy chain IIA	30% (15 years)
CFHR5 nephropathy	*CFHR5* Heterozygous (males) Heterozygous (females)	Complement factor H-related protein 5	80% (49 years) 20% (56 years)
Glomerulopathy associated with fibronectin deposition	*FN1*	Fibronectin 1	>90% (2nd to 6th decade)

*ESKD: End stage kidney disease
**TBMN: Thin basement membrane nephropathy

glomerulopathy, which is characterized by deposition of complement 3 (C3) without immunoglobulins, low serum C3 levels but normal C4 levels [38]. This comprises the clinical conditions known as dense deposit disease, C3 glomerulonephritis, and complement factor H-related 5 (CFHR5) nephropathy [38–40]. Mutations in *CFH*, *CFI*, *C3*, and *CFHR5* genes encode proteins that regulate the alternative complement pathway. Studies from families of Cypriot descent have shown that patients with duplication of exons 2–3 of the *CFHR5* gene present with hematuria, proteinuria, and hypertension, with up to 50% progressing to end-stage kidney failure within 10 years of diagnosis [40]. Another rare cause of familial hematuria is the autosomal dominant MYH9 (ADM9) spectrum disorders caused by mutations in the *MYH9* gene encoding for the nonmuscle myosin heavy chain IIA [41, 42]. Extrarenal symptoms include macrothombocytopenia, sensory hearing loss, congenital cataract, and leukocyte Döhle bodies in neutrophils. The associated nephropathy seen in 30–70% of patients presents initially with microscopic hematuria with proteinuria developing as the disease progresses, and finally reaching end-stage kidney failure in young adulthood. De novo *MYH9* gene mutations may occur in 35%. Fibronectin gene mutations also result in an autosomal dominant glomerulopathy associated with fibronectin deposits [43, 44]. Clinical features include microscopic hematuria, proteinuria, and hypertension with progression to end-stage kidney disease by the second to sixth decade of life [45].

Clinical Approach (Figs. 1 and 2)

A comprehensive history will help in elucidating the cause of the hematuria. Points to ask in the history include the color of the urine, timing of color change in relation to urinary stream, and associated signs, symptoms, illness, or activity. In girls at the peripubertal age, menarche as a cause of hematuria should be considered. Transient hematuria has been found in association with fever, exercise, urinary tract infections (which usually also present with dysuria and pyuria),

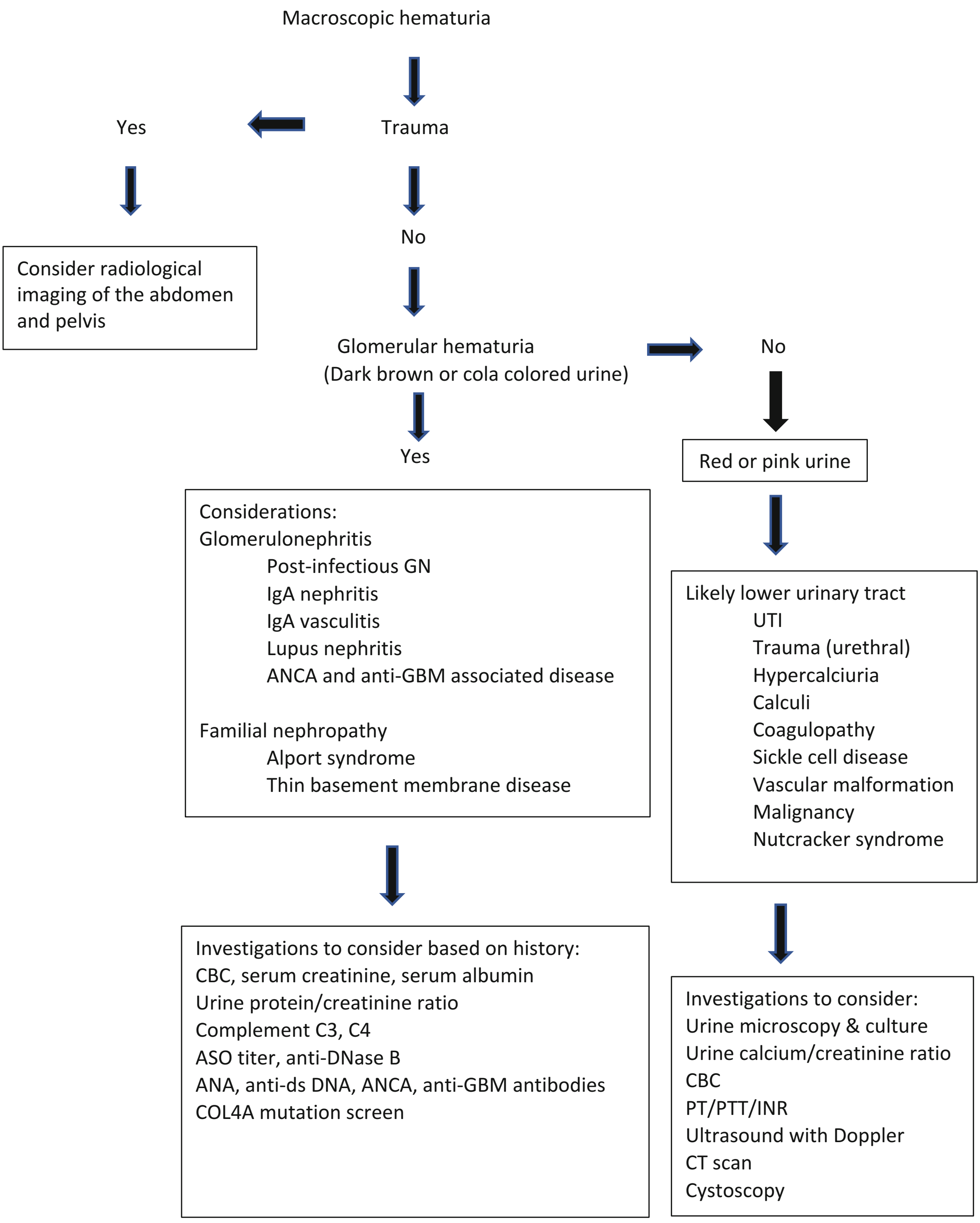
Macroscopic hematuria
Trauma
Yes
Consider radiological imaging of the abdomen and pelvis
No
Glomerular hematuria (Dark brown or cola colored urine)
Yes
No
Red or pink urine
Considerations:
Glomerulonephritis
Post-infectious GN
IgA nephritis
IgA vasculitis
Lupus nephritis
ANCA and anti-GBM associated disease
Familial nephropathy
Alport syndrome
Thin basement membrane disease
Likely lower urinary tract
UTI
Trauma (urethral)
Hypercalciuria
Calculi
Coagulopathy
Sickle cell disease
Vascular malformation
Malignancy
Nutcracker syndrome
Investigations to consider based on history:
CBC, serum creatinine, serum albumin
Urine protein/creatinine ratio
Complement C3, C4
ASO titer, anti-DNase B
ANA, anti-ds DNA, ANCA, anti-GBM antibodies
COL4A mutation screen
Investigations to consider:
Urine microscopy & culture
Urine calcium/creatinine ratio
CBC
PT/PTT/INR
Ultrasound with Doppler
CT scan
Cystoscopy

Fig. 1 Flow chart of approach to macroscopic hematuria

and trauma. Lower urinary tract origin of hematuria is associated with passage of fresh blood with or without clots. Hematuria occurring at the beginning of the urinary stream is seen in urethral causes such as urethritis, whereas terminal hematuria is indicative of a bladder cause such as bladder calculus or tumors and schistosomiasis. This is usually accompanied by lower urinary tract symptoms such as frequency, urgency, and dysuria. In addition, a history of sexual activity

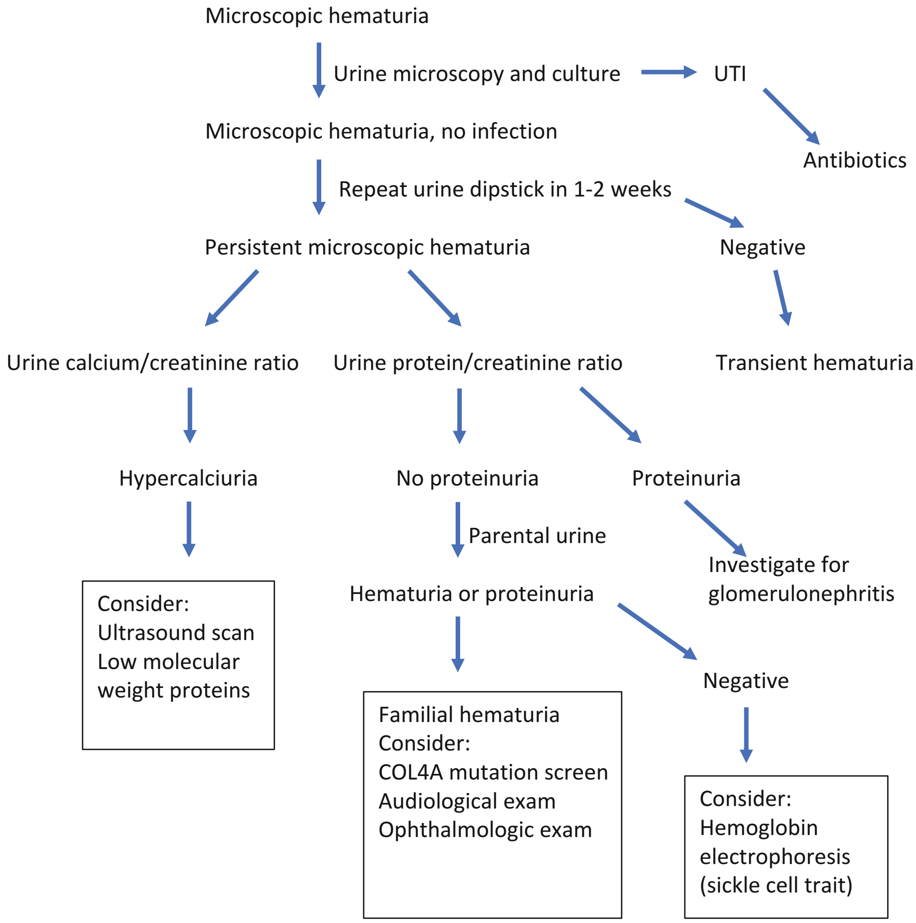
Microscopic hematuria
Urine microscopy and culture
UTI
Antibiotics
Microscopic hematuria, no infection
Repeat urine dipstick in 1-2 weeks
Negative
Persistent microscopic hematuria
Transient hematuria
Urine calcium/creatinine ratio
Urine protein/creatinine ratio
Hypercalciuria
No proteinuria
Proteinuria
Parental urine
Investigate for glomerulonephritis
Hematuria or proteinuria
Consider: Ultrasound scan Low molecular weight proteins
Negative
Familial hematuria Consider: COL4A mutation screen Audiological exam Ophthalmologic exam
Consider: Hemoglobin electrophoresis (sickle cell trait)

Fig. 2 Algorithm for evaluation of microscopic hematuria

should be obtained in adolescents. The presence of flank pain or abdominal pain could suggest hydronephrosis, urolithiasis, nutcracker syndrome, or hemorrhage in polycystic kidney disease. Detection of ballotable kidneys and palpable bladder on examination will aid in the diagnostic evaluation.

Children with acute glomerulonephritis present with cola-colored or dark brown urine associated with edema and hypertension, accompanied by a latent history of upper respiratory tract infection 7 to 10 days or skin infection 2 to 3 weeks prior to onset of hematuria. Recurrent episodes of gross hematuria occur in many of the glomerulopathies, in particular IgA nephropathy and the other familial hematuria syndromes. Evaluation for extrarenal symptoms such as hearing loss and ocular abnormalities should be performed. Systemic signs to look for on physical examination include fever, pallor, bruising, rashes, joint swelling, mouth ulcers, hypertension, hepatosplenomegaly, and lymphadenopathy.

Evaluation of the family history not only of hematuria but in particular onset of end-stage kidney disease will often give a clue as to the underlying diagnosis. In addition to the familial hematuria syndromes, other conditions associated with a positive family history include stone disease and polycystic kidney disease.

The first step in the investigation is to determine the presence of hematuria. Urine dipstick examination has been used as a screening tool to detect hematuria. This test is based on the pseudoperoxidase activity of hemoglobin to catalyze the reaction between heme and tetramethylbenzidine resulting in a green-blue color due to the oxidized chromogen. These reagent strip tests are highly sensitive and can detect as low as five red blood cells per microliter or 150 microgram per liter of free hemoglobin. Causes of false positive results include hemoglobinuria, myoglobinuria, and presence of oxidizing agents in the urine such as vegetable peroxidases and microbial peroxidases associated with bacterial contamination of the urine [46]. Causes of false negative results include presence of large amounts of reducing agents such as ascorbic acid and high urine–specific gravity, which causes crenation of red blood cells preventing lysis.

Confirmation of hematuria by urine microscopic examination demonstrates the presence of red blood cells of more than 5 per microliter in a fresh uncentrifuged midstream urine specimen or more than 3 red blood cells per high-power field in the centrifuged sediment from 10 ml of freshly voided midstream urine [2]. False negative results can occur when the urine is of low specific gravity or is alkaline as this causes red blood cell lysis. The presence of red cell casts in fresh urine is indicative of a glomerulonephritis. These disintegrate in alkaline urine, appearing as granular casts. In children with asymptomatic microscopic hematuria, further evaluation is indicated if the hematuria is persistent, that is, present in two out of three consecutive samples.

The next step in evaluating the child with hematuria is to determine if this is due to glomerular or nonglomerular origin. The presence of greater than 30% dysmorphic red blood cells, in particular acanthocytes on urine phase contrast microscopy, is indicative of glomerular hematuria, whereas greater than 90% of isomorphic cells are seen in nonglomerular hematuria [47, 48]. Accompanying significant proteinuria of greater than 20 mg/mmol (0.2 g/g creatinine) in children older than 2 years of age and 50 mg/mmol (0.5 g/g creatinine) in younger children is also suggestive of an underlying glomerulopathy. Proteinuria may be detected in patients with macroscopic hematuria, but is usually less than 2+ (1 g/L) on dipstick examination.

In children with nonglomerular hematuria, urine culture should be sent especially if there are lower urinary tract symptoms or fever. Gross hematuria associated with lower urinary tract symptoms is suggestive of hemorrhagic cystitis, and urine should be sent for adenovirus culture. BK virus infection should also be considered in immunocompromised patients with cystitis. In endemic areas, the urine should be examined for *Schistosomia hematobium* ova.

As hypercalciuria is a common cause of nonglomerular hematuria, the spot urine calcium to creatinine ratio should be assessed. A value greater than 0.6 mmol/mmol (0.2 mg/mg creatinine) in children 2 years and older, and greater than 1.7 mmol/mmol (0.6 mg/mg creatinine) in infants between the ages of 6 and 12 months is indicative of hypercalciuria [49, 50]. If there is accompanying abdominal pain, in particular loin to groin pain suggestive of ureteric calculi, a plain abdominal radiograph is indicated. Ultrasound scan of the kidneys and bladder is indicated in nonglomerular hematuria to exclude congenital malformations including cystic kidney disease, renal calculi, and tumors.

Doppler ultrasound of the left renal vein is useful to exclude nutcracker syndrome in children with asthenic habitus or who have recurrent left flank pain associated with hematuria. Nutcracker syndrome is detected by Doppler ultrasonographic assessment of the left renal vein diameter and peak velocity. Suggested ultrasonographic criteria for diagnosis include an anteroposterior diameter ratio between the distended and narrowed portions of the left renal vein of greater than 4.0, and peak velocity ratio between the narrowed and distended portions of the left renal vein of greater than 4.2 [25, 51]. Axial imaging by computed tomography or magnetic resonance imaging may be required when Doppler renal vein ultrasonography is not diagnostic [25, 26]. The angle between the superior mesenteric artery and abdominal aorta should be less than 35° to diagnose nutcracker syndrome. Compression of the left renal vein in the fork between the superior mesenteric artery and abdominal aorta results in a left renal vein hilar to aortomesenteric diameter ratio of greater than 4.9 accompanied by gonadal vein distension and pelvic congestion. The “beak” sign is the most diagnostic on computed tomography imaging, and is due to severe narrowing of the left renal vein at the aortomesenteric portion [52].

Coagulation screen is necessary if there is a history suggestive of a bleeding disorder. Finally, cystoscopy is indicated in nonglomerular hematuria if a bladder or urethral pathology is suspected due to accompanying voiding symptoms, or when a mass is detected on bladder ultrasound, or to lateralize the source of bleeding.

In children with glomerular hematuria, renal function assessment in the form of blood urea and serum creatinine is required. Serum albumin should be measured if there is significant proteinuria. Subsequent investigations are tailored according to the type of clinical presentation. As the familial hematuria syndromes are an important cause of hematuria in children, urine screening of the family is useful. Audiometry to detect high-frequency sensorineural hearing loss and screening for ophthalmologic abnormalities may be indicated in patients with suspected Alport syndrome. If the rare MYH9 spectrum of disorders is suspected, a peripheral blood smear will be useful to look for macrothrombocytopenia and basophilic cytoplasmic leukocyte inclusion bodies (Döhle-like bodies). Genetic analysis for the relevant mutations associated with the familial hematuria syndromes is recommended in the presence of a positive family history or suggestive clinical, laboratory, or biopsy features.

Evaluation of children with suspected glomerular pathology should include serum complements C3 and C4, anti-streptolysin O titers (ASOT) or anti-DNAse B antibody, anti-nuclear antibodies (ANA), anti-double-stranded DNA (dsDNA) antibody, anti-neutrophil cytoplasmic antibodies (ANCA) in particular anti-proteinase 3 and anti-myeloperoxidase antibodies, IgA levels, hepatitis B surface antigen, and viral titers if appropriate. Kidney ultrasound is useful in the evaluation of a patient with glomerular hematuria to assess chronicity of the glomerulonephritis by determining kidney size and echogenicity.

Kidney biopsy is indicated in those with significant proteinuria (>1 g/1.73m^2/day) except in post-infectious glomerulonephritis, persistently low serum complement C3, unexplained kidney failure, family history of significant kidney disease suggestive of Alport syndrome where genetic analysis is not available, and recurrent gross hematuria of unknown etiology.

Approach to the Child with Proteinuria

Introduction

Proteinuria, like hematuria, is one of the most common reasons why a child is referred to a pediatric nephrologist. The presentation of a child with proteinuria can range from incidental detection of protein following urine dipstick testing during a routine medical visit in an asymptomatic child to presentation with generalized edema and nephrotic syndrome. The presence of glomerular proteinuria is often a sign of significant disease that needs further evaluation and treatment. However, there are a few situations in children where proteinuria can be transient or physiological such as during a febrile illness or following exercise.

Proteinuria is a general term which refers to increase loss of plasma proteins in the urine. This can be either due to: (1) increased glomerular permeability to large-molecular-weight proteins (for example albumin in glomerular diseases such as nephrotic syndrome), (2) incomplete tubular reabsorption of low-molecular-weight proteins (for example beta 2 microglobulin and retinol binding protein in renal tubular disorders such as cystinosis and Dent disease), and (3) increased plasma production of low-molecular-weight proteins (such as immunoglobulin-free light chains in monoclonal gammapathies and chronic inflammatory conditions).

Albuminuria Versus Proteinuria

Albuminuria refers to increased albumin excretion in the urine and has been increasingly used instead of proteinuria as a marker of chronic kidney disease in adults. The KDIGO (Kidney Disease: Improving Global Outcomes) 2012 clinical practice guideline for the evaluation and management of chronic kidney disease has incorporated albuminuria in the classification of chronic kidney disease in adults [53]. In health, <30% of the total protein in the urine is albumin, with Tamm–Horsfall protein (secreted by the ascending limb of loop of Henle) making up approximately 50% and the rest includes small quantities of immunoglobulins, transferrin and beta-2 globulin [54]. However, as protein losses increase albumin becomes the predominant protein in the urine [55]. In addition, urinary albumin measurement is technically superior to urine protein measurement. Some authors have, therefore, strongly recommended measuring albuminuria only for assessing chronic kidney disease [55, 56]. Although albuminuria appears to be increasingly adopted as an early marker of chronic kidney disease in adults, recent guidelines for children [57] recommend measuring proteinuria rather than albuminuria [53, 54, 58]. Although, one can miss the occasional child with tubular proteinuria when measuring albuminuria to detect proteinuria, urine total protein assessment is not a reliable marker for tubular proteins, and one should specifically measure urine low molecular proteins such as beta 2 microglobulin and retinol binding protein if renal tubular disease is suspected.

How to Measure Urine Protein

Urine dipstick: Urine reagent strip devices (dipsticks) are commonly used as a screening test for proteinuria. It is a semi-quantitative test where the test pad changes in color depending on the concentration of albumin in the urine [59]. The protein pad does not detect other proteins such as gamma globulins or Bence Jones proteins. The results are affected by the concentration and the pH of the urine. An alkaline or concentrated urine sample can lead to false positives and conversely acidic or dilute samples can result in false negatives. The dipstick will also be falsely negative when there is non-albumin proteinuria such as in renal tubular disorders. In general, 1+ protein on most dipstick is approximately 330–669 mg/l of protein (urine protein: creatinine ratio [urine PCR] 50-99 mg/mmol or 0.5–0.99 g/g), 2+ is 1000–2000 mg/l (100–450 mg/mmol or 1–4.5 g/g), and 3+ is >3000 mg/l (>450 mg/mmol or >4.5 g/g) [55]. A number of international guidelines do not recommend use of dipstick testing for detection of proteinuria because of the high false negative and poor negative predictive value compared to laboratory testing [55, 57]. Therefore, where accurate quantification of proteinuria is required, a urine sample should be sent to the laboratory for a timed collection measurement or for urine albumin:creatinine ratio (ACR) or PCR.

Spot Urine Sample Versus 24-h Collection

Although 24-h urine collection was previously the gold standard for assessment of proteinuria, this has largely been replaced by spot urine samples. A number of studies have shown that the spot urine protein (or albumin): creatinine ratio provides an accurate estimation of protein excretion rate [60]. It is also more convenient to obtain when compared to 24-h urine collection. Therefore, spot urine samples should be utilized to detect and monitor proteinuria in children [58].

Definition: The definition of proteinuria depends on the age of the child. Neonates have higher levels of protein in the urine, particularly in the first 2 weeks of life. In preterm infants this is significantly higher and predominantly composed of tubular proteins [61]. Beyond 6 months and up to 24 months urine PCR of <50 mg/mmol or 0.5 g/g is considered normal. In children over 2 years a urine PCR of <20 mg/mmol (0.2 g/g) is normal. If a 24 hour urine collection is performed, a protein excretion of <100 mg/m^2/24 h is normal [54]. Generally, nephrotic-range proteinuria is defined by a urine PCR > 200 mg/mmol (0.2 g/g) in a spot sample and in a timed or 24-h collection >40 mg/m^2/h or 3 g/1.73m^2/day. Microalbuminuria is defined by a urine ACR >3 mg/mmol (>0.3 g/g) in a first morning urine sample; the standard dipstick test does not detect this level of proteinuria.

Clinical Approach

Proteinuria is a relatively common problem. In a mass school screening program in Japan involving over 380,000 children aged 6–14 years, isolated proteinuria was detected in a first morning urine sample in 0.62% of children 6–11 years of age and 2.3% in 12–14 year age group [4]. However, upon repeat testing, the positive rates dropped to 0.08% in the younger group and 0.37% in the older age group. The study found that asymptomatic urinary abnormalities were 10 times more frequent than significant pathology in those children who had histological evaluation, demonstrating the unacceptably high sensitivity of mandatory urine screening program in asymptomatic school children.

Once proteinuria is detected on a dipstick test, it is important to establish if this is pathological or nonpathological proteinuria. Table 3 lists the causes of proteinuria.

Nonpathological Proteinuria

In many children, proteinuria is transient and is not present in all the urine samples tested. This may be related to an inter-current illness or posture. Repeating the test when the child has recovered from the illness or performing the test on a first morning sample helps clarify the situation. Children with intermittent or transient proteinuria do not require detailed investigations and there is no evidence to suggest that this has any prognostic significance.

(a) Febrile proteinuria: Proteinuria is often detected in children without underlying kidney disease during a febrile illness, usually where the temperature is >38.4 °C [62]. Resolution of proteinuria when the test is repeated following recovery from the acute illness supports the diagnosis. While no explanation is available for this phenomenon, it is likely that the state of hydration, plasma volume, and stress hormones such as adrenaline might have a role to play.

(b) Exercise-induced proteinuria: Proteinuria is well recognized after strenuous exercise. This can range from normal physiological excretion to up to 120 mg/hr. The type of physical activity appears to influence the amount of proteinuria, with subjects engaged

Table 3 Causes of proteinuria

A) Non-pathological proteinuria	B) Pathological proteinuria	
1. Febrile proteinuria 2. Exercise induced proteinuria 3. Orthostatic proteinuria	1. Glomerular proteinuria	2. Tubular proteinuria
	a) Primary glomerular disease Idiopathic childhood nephrotic syndrome Focal segemental glomerulosclerosis Mesangiocapillary glomerulonephritis Congenital nephrotic syndrome Membranous nephropathy	a) Inherited Cystinosis Lowe syndrome Galactosemia Mitochondrial cytopathies Dent disease Wilson disease Tyrosinaemia Hereditary fructose intolerance
	b) Secondary glomerular disease Post-infectious glomerulonephritis IgA vasculitis Lupus nephritis Alport syndrome Hepatitis B and C Human immunodeficiency virus Amyloidosis	b) Acquired Drug induced Pyelonephritis Acute tubular necrosis Heavy metal poisoning
	c) Other glomerular diseases Hemolytic uremic syndrome Hypertension Diabetes mellitus Hyperfiltration	

in maximal short-term running excreting more proteins when compared to swimming, bicycling, or rowing, despite engaging in similar intensity of exercise as demonstrated by post-exercise lactate levels [63]. The mechanism of exercise-induced proteinuria is thought to be renal vasoconstriction and consequently decreased glomerular filtration rate (GFR) along with increased glomerular permeability and impaired protein reabsorption [63]. A normal first morning urine PCR 48 hours after the intense exercise would help support the diagnosis of exercise-induced proteinuria. While evidence in children is lacking, data from adult studies do not suggest that this transient proteinuria is pathological.

(c) Orthostatic proteinuria: This is one of the most common causes of nonpathological proteinuria, with a prevalence of 0.65–5% reported in children on initial screening. The prevalence may be less if the first, second, and third random urine samples are checked at 2 weeks intervals following the initial detection of asymptomatic proteinuria as this method is more likely to exclude other causes of transient proteinuria [64]. Orthostatic proteinuria is more often seen in thin children compared to those who are overweight. The peak incidence is in adolescence and it is rare after the age of 30 years. The mechanism for orthostatic proteinuria has not been clearly defined. Since it is more commonly seen in children with low BMI and the prevalence reduces with increasing age and concomitant increase in adiposity, an exaggerated response to upright posture with or without anatomical factors such as renal vein entrapment has been postulated [64]. In those children with persistent proteinuria on a random sample, a first morning sample should be sent to measure urine PCR. A normal first morning urine PCR in a child with proteinuria on a random urine sample would confirm a diagnosis of orthostatic proteinuria. The long-term prognosis appears to be excellent with resolution of the proteinuria in the vast majority of affected patients during adulthood. Although some would recommend follow-up of these children, it does not seem justifiable in the absence of coexisting hematuria, hypertension, or impaired kidney function.

Pathological Proteinuria

Pathological proteinuria is most often glomerular in origin. The mechanism of proteinuria can vary depending upon the underlying pathology. For instance, in minimal change disease there is loss of negative charge in the glomerular filtration barrier, leading to selective albuminuria and very little increase in the glomerular permeability to globulins [65]. Mutations in the slit diaphragm proteins such as nephrin and podocin lead to absence or malfunction of the slit diaphragm, and consequently nonselective proteinuria [66]. In patients with reduced nephron mass such as children with scarred kidneys and in kidney donors there is increase in glomerular permeability to plasma proteins in the residual nephrons, leading to proteinuria. Another mechanism is due to changes in glomerular capillary pressure secondary to disease such as hyperfiltration in diabetic nephropathy, resulting in increased filtration fraction.

Tubular proteinuria is most often due to inherited or acquired damage to the proximal convoluted tubule. The predominant proteins in the urine in this instance are low-molecular-weight proteins such as β2 microglobulin and retinol-binding protein rather than albumin. The degree of proteinuria is usually <1 g/1.73m^2/day. Secretory proteinuria is seen in neonates due to excretion of Tamm–Horsefall protein. Monoclonal gammopathy or multiple myeloma can lead to overflow proteinuria due to increased production which can exceed the maximal tubular reabsorption for that protein.

Child presenting with persistent proteinuria: Nephrotic-range proteinuria and nephrotic syndrome are dealt with in a separate chapter and so will not be discussed here. We will discuss the evaluation of child with subnephrotic-range proteinuria – urine PCR 50-200 mg/mmol (0.5–2.0 g/g) in a spot sample or <40 mg/m^2/h (<3 g/1.73m^2/day) in a timed collection.

History: It is important to ask about any recent illness and inquire about fluid intake and urine output. Document any history of visible blood in the urine. Ask about any facial or lower limb swelling. Connective tissue disorder symptoms such as rash, joint pain, or swelling should be noted. Documentation of any drug intake, including nonsteroidal anti-inflammatory agents and antibiotics, is essential. Family history of connective tissue disease, deafness, or CKD should be obtained.

Physical examination: The general health of the child as to whether he/she is well or not should be noted. Blood pressure should be measured. Look for any dysmorphic features such as microcoria (Pierson syndrome) or ambiguous genitalia (Denys-Drash syndrome). Hypotonia is a feature of Galloway Mowat syndrome and also Pierson syndrome. Assess the peripheral circulation by checking the capillary refill time and also noting whether the hands and feet are warm or cool. Inspect for any vasculitic rash and joint swelling (IgA vasculitis and lupus nephritis). Examine for the presence of ankle edema, genital swelling, ascites, and pleural effusion. It is important to assess for signs of fluid overload such as raised jugular venous pulse, hepatomegaly, and gallop rhythm.

Investigations (Fig. 3): Detailed investigations are only indicated once persistent proteinuria has been confirmed. Therefore, the first step in the evaluation of a child referred with proteinuria is to establish if this is persistent or not. In those children found to have + or ++ of protein on dipstick test during an acute illness, repeating the test a couple of weeks following the illness will ensure that children with transient proteinuria are not unnecessarily investigated. Those children with persistent proteinuria of 1+ or more should have a first morning urine sample sent for urine PCR. If the result is >20 mg/mmol (>0.2 mg/mg), further evaluation is indicated. Requesting first morning urine PCR (ideally on 3 consecutive days) will also identify those children with orthostatic proteinuria.

Urine investigations: Dipstick analysis of the urine provides valuable information including the presence or not of blood, white cells, and nitrites. It also provides information about the specific gravity and pH of the urine. Urine microscopy and culture is important in all children presenting with proteinuria to look for the presence of hematuria, pyuria, casts, and crystals. It also helps identify any undiagnosed urinary tract infection. Urine tubular proteins such as β2 microglobulin and retinol binding protein should be requested if a tubular disorder is suspected.

Blood investigations: Assessment of kidney function by measuring plasma creatinine along with urea and electrolytes is recommended. The Schwartz formula [67] should be utilized to calculate the estimated GFR. A reduced eGFR is an indication for performing a kidney biopsy. Measuring plasma albumin along with liver function tests is important as this will show if the proteinuria has led to drop in the plasma levels of albumin. It usually takes more than a few days of nephrotic-range proteinuria for the plasma albumin to drop to <30 g/l. In a child with proteinuria with or without hematuria, a low plasma albumin is an indication to perform a kidney biopsy. A lipid profile is often checked in children with nephrotic syndrome as it will be raised. However, there is no indication to treat this in the acute phase.

Complement C3 and C4 should be checked in all children with persistent proteinuria without an obvious cause. Post-infectious glomerulonephritis, shunt nephritis, and C3 glomerulopathy are associated with low C3 while low C3 and C4 are seen in lupus nephritis. Antinuclear antibodies (ANA), anti-double-stranded DNA antibodies (dsDNA), anti-streptolysin O titers (ASOT) or anti-DNase B titers, and anti-neutrophil cytoplasmic antibodies (ANCA) ought to be requested if there are any indication of glomerulonephritis. In certain children where the history and the initial investigations are suggestive of a chronic illness, human immunodeficiency virus (HIV) and hepatitis B and C virus screen may be indicated. In addition, a blood sample may be indicated for gene mutational studies, particularly if inherited cause of proteinuria is suspected based on the family history or clinical examination.

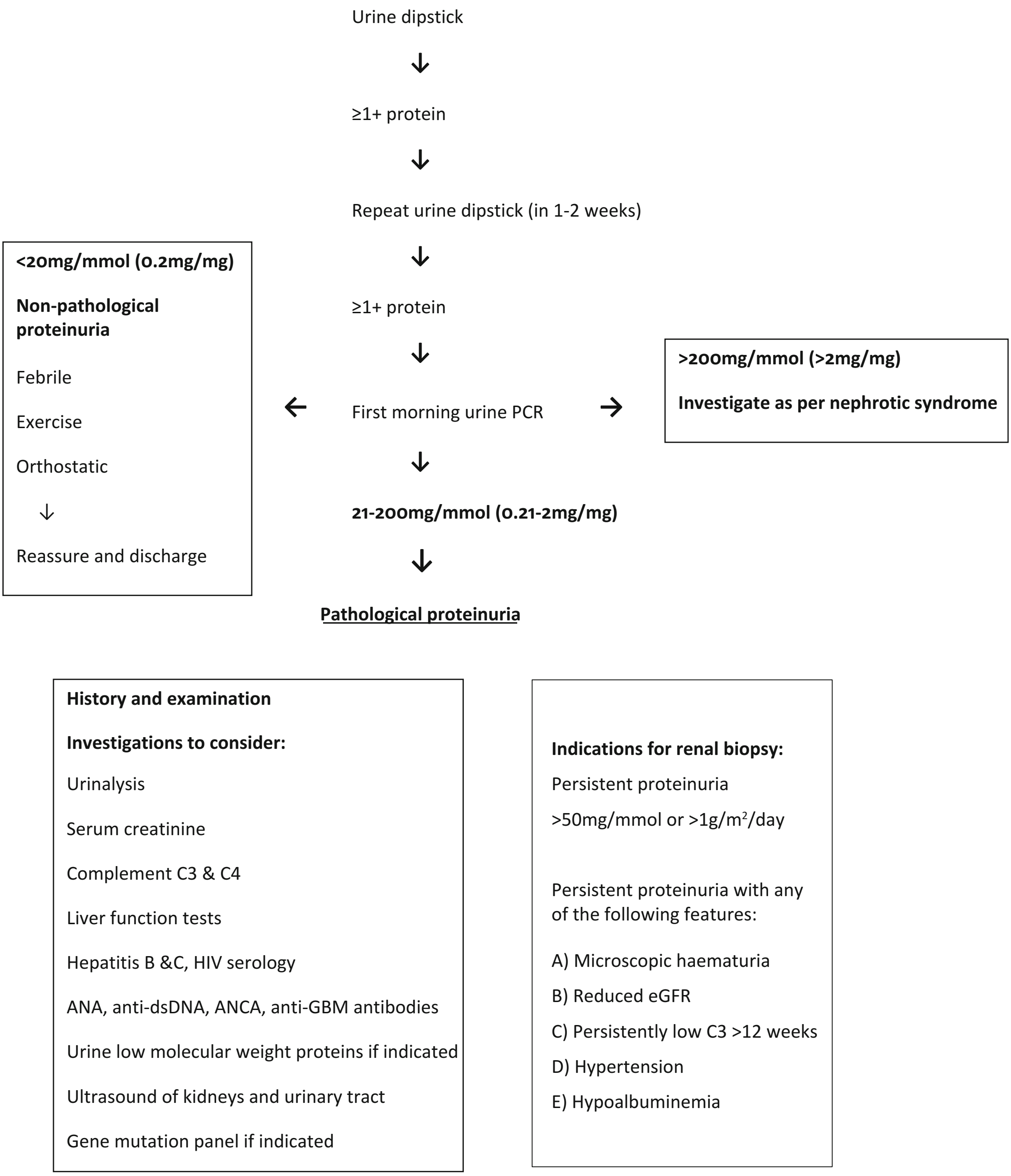
Urine dipstick
≥1+ protein
Repeat urine dipstick (in 1-2 weeks)
≥1+ protein
First morning urine PCR
<20mg/mmol (0.2mg/mg)
Non-pathological proteinuria
Febrile
Exercise
Orthostatic
Reassure and discharge
>200mg/mmol (>2mg/mg)
Investigate as per nephrotic syndrome
21-200mg/mmol (0.21-2mg/mg)
Pathological proteinuria
History and examination
Investigations to consider:
Urinalysis
Serum creatinine
Complement C3 & C4
Liver function tests
Hepatitis B &C, HIV serology
ANA, anti-dsDNA, ANCA, anti-GBM antibodies
Urine low molecular weight proteins if indicated
Ultrasound of kidneys and urinary tract
Gene mutation panel if indicated
Indications for renal biopsy:
Persistent proteinuria
>50mg/mmol or >1g/m²/day
Persistent proteinuria with any of the following features:
A) Microscopic haematuria
B) Reduced eGFR
C) Persistently low C3 >12 weeks
D) Hypertension
E) Hypoalbuminemia

Fig. 3 Algorithm for evaluation of child with proteinuria

Imaging investigations: Ultrasonography of the kidneys is routinely performed in the evaluation of most children with proteinuria in order to exclude any structural abnormalities such as small kidneys due to loss of nephron mass, which can be congenital or acquired. Nuclear medicine scans are useful in identifying scarring in the kidneys where proteinuria due to hyperfiltration is suspected. Doppler ultrasound is suggested for identifying renal artery stenosis; however, CT/MR angiogram and often digital subtraction angiography is required to diagnose these conditions.

Indications for Kidney Biopsy in a Child with Proteinuria (Fig. 3)

Persistent proteinuria: A kidney biopsy should be considered in any child with urine PCR >50 mg/mmol or 0.5 g/g (>1 g/1.73m^2/day) for more than 4 weeks. The presence of hematuria along with proteinuria also greatly increases the chance that a biopsy will lead to a diagnosis. A study from Korea found that IgA nephritis was the most common diagnosis made in children with combined proteinuria and hematuria initially identified through population screening [5]. A reduced eGFR is another indication for kidney biopsy. Post-infectious glomerulonephritis is an exception to this rule, as in the overwhelming majority of children with this condition there is spontaneous recovery of kidney function in a few weeks. A persistently low complement C3 level beyond 3 months is another indication for kidney biopsy as this often suggests immune complex or C3 glomerulopathy. Persistent proteinuria in a systemic condition such as lupus nephritis or IgA vasculitis merits a biopsy to help stage the condition and plan treatment.

Management of children with mild proteinuria: It is well recognized that glomerular proteinuria may play a role in progression of CKD. Proteinuria has also been linked to be a risk factor for cardiovascular diseases in adults. High levels of proteinuria can lead to complications such as hyperlipidemia, infection, and thromboembolism. It is therefore important that proteinuria is detected and where possible, specific treatment should be promptly instituted.

In those children where the urine PCR is between 20–50 mg/mmol or 0.2–0.5 g/g (300–1000 mg/1.73m^2/day) and the rest of the investigations have not identified a pathology, it is debatable whether to proceed to a kidney biopsy as the chances of identifying a progressive disease is small and also because this may not alter the clinical management of the child. It is recommended that these children have 6–12 monthly assessment of proteinuria, blood pressure, and plasma creatinine. While reduction of proteinuria using angiotensin converting enzyme inhibitors (ACEi) has been shown to reduce the progression of chronic kidney disease in children [68, 69], there are no such data to support this treatment in those without chronic kidney disease. There is also the concern that progressive disease might be masked by treating with an anti-proteinuric medication before a diagnosis has been established. In view of this, regular monitoring of the clinical situation is indicated and proceeding to a kidney biopsy if there are any signs of disease progression. Following this, ACEi treatment can be considered for persistent proteinuria even in situations where a clear diagnosis has not yet been established. Tubular proteinuria is not thought to have a prognostic significance. The role of ACEi in reducing tubular proteinuria and protecting kidney function has not been established.

Cross-References

- ▶ Acute Post-Streptococcal Glomerulonephritis
- ▶ Henoch Schönlein Purpura Nephropathy
- ▶ Immunoglobulin A Nephropathy in Children
- ▶ Inherited Diseases of the Glomerular Basement Membrane
- ▶ Lupus Nephritis
- ▶ Membranoproliferative Glomerulonephritis and C3 Glomerulopathy in Children
- ▶ Nephrotic Syndrome: Classification and Evaluation
- ▶ Pediatric Fanconi Syndrome
- ▶ Pediatric Renal Tumors
- ▶ Pediatric Tubulointerstitial Nephritis
- ▶ Renal Manifestations of Metabolic Disorders in Children
- ▶ Renal Vasculitis
- ▶ Urinary Tract Infections in Children

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Part III

Glomerular Diseases

Nephrotic Syndrome: Classification and Evaluation

9

Patrick Niaudet

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Abstract

Nephrotic syndrome is defined by massive proteinuria, hypoalbuminemia, edema, and hyperlipidemia. Proteinuria is secondary to a defect of the glomerular filtration barrier. The main complications of nephrotic syndrome are infections and thromboembolic events. Although nephrotic syndrome may be secondary to many renal diseases, idiopathic nephrotic syndrome is the most frequent cause in childhood with a complete remission following corticosteroid therapy in 90% of cases. Less frequently, nephrotic syndrome is due to a primary or secondary glomerulonephritis or to a genetic defect of podocyte or glomerular basement membrane proteins. Genetic testing is proposed in children with steroid-resistant idiopathic nephrotic syndrome, children less than 1 year, children with familial history of proteinuria, or those with extrarenal symptoms. Kidney biopsy is performed in children older than 12 years and children who do not respond to steroid therapy.

Keywords

Nephrotic syndrome · Minimal change disease · Focal segmental glomerulosclerosis · Genetic testing · Kidney biopsy

Definition

Nephrotic syndrome is defined by the presence of massive proteinuria (>50 mg/kg/day or >40 mg/hour/m^2 or urine protein/creatinine ratio >200 mg/

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mmol), hypoalbuminemia (<30 g/l), peripheral edema, and hyperlipidemia.

The presence of edema is inconstant and does not always correlate with the serum albumin concentration. As fluid retention is primarily due to increased sodium reabsorption at the level of the collecting duct by the epithelial sodium channel (ENaC) [1], poor salt intake, for example, may explain the absence of edema. Urinary protein excretion may fall in patients with severe nephrotic syndrome when plasma albumin is very low.

Pathogenesis

Proteinuria in nephrotic syndrome is due to an increased filtration of macromolecules across the glomerular capillary wall. The glomerular filtration barrier is composed of three components: fenestrated endothelial cells, the glomerular basement membrane (GBM), and podocytes. The structure of the GBM comprises a central electron-dense layer, the lamina densa, an electron-lucent lamina rara interna on the endothelial cell side, and a lamina rara externa on the podocyte side [2]. The GBM is composed of type IV collagen, laminin, nidogen, and heparan sulfate proteoglycans, such as agrin [3]. Podocytes are highly specialized epithelial cells in contact with lamina rara externa through the foot processes [4]. Foot processes of adjacent podocytes interdigitate with 30–40 nm gaps that are maintained by the slit diaphragm, a zipper-like pattern membrane.

The filtration of macromolecules across the glomerular capillary wall is normally restricted by two mechanisms: charge-selectivity and size-selectivity [5, 6]. The endothelial cells and the GBM have a net negative charge due to polyanions such as heparan sulfate proteoglycans. This creates a charge barrier to filtration of large anions such as albumin. In minimal change disease, the most common cause of nephrotic syndrome in children, there is a loss of anionic charge that is not accompanied by any structural damage or change to the glomerular filtration unit observed by light microscopy [7]. However, electron microscopy demonstrates epithelial foot process effacement.

The glomerular capillary wall is size-selective, having functional pores of approximate radius of 40–45 A (radius of albumin is ~36 A) [6]. These pores appear to be located throughout the GBM and across the GBM at the slit diaphragms between adjacent epithelial cell foot processes. In comparison, the width of the endothelial cell fenestrae is much larger (375–400 A). As a result, the endothelial cell is not part of the size barrier to macromolecule filtration. In glomerular diseases other than minimal change disease, structural injury seen by light microscopy results in increase in the number of large pores in the GBM. This structural damage allows movement of normally restricted proteins of varying sizes (including large neutral proteins, such as IgG) across the filtration barrier.

Several mechanisms may lead to a glomerular injury and the nephrotic syndrome. In minimal change disease and idiopathic focal segmental glomerulosclerosis (FSGS), circulating factors alter podocyte function leading to proteinuria [8, 9]. Such circulating factors explain the immediate recurrence of proteinuria after kidney transplantation in patients who have progressed to end-stage renal disease. Immune mechanisms explain structural changes of the GBM secondary to in situ formation of immune complexes as in membranous nephropathy [10], in situ deposition of immune complexes as in lupus nephritis [11], and direct effects of anti-GBM antibodies as in anti-GBM disease [12]. Third, mutations of podocyte or slit diaphragm proteins leading to structural or functional defects of podocytes are observed in inherited forms of congenital syndrome, in ~30% of patients with steroid-resistant idiopathic nephrotic syndrome, and in some syndromic forms of nephrotic syndrome [13].

Clinical Evaluation at Onset

Clinical and laboratory evaluation are used to confirm the diagnosis and determine the underlying cause. The child should be examined to detect peripheral edema, ascites, or pleural or pericardial effusion. Blood pressure should be measured as hypotension may be related to hypovolemia, whereas hypertension may be related to glomerulonephritis. Physical examination is important to detect infection or thrombosis as a complication of the nephrotic syndrome.

The diagnosis of nephrotic syndrome is confirmed by the presence of both nephrotic-range proteinuria and hypoalbuminemia. Nephrotic-range proteinuria is defined as urine protein excretion greater than 50 mg/kg per day or 40 mg/m^2 per hour. It is higher at onset and decreases as plasma albumin concentration falls. However, it is difficult to obtain accurately timed urine collections in young children. An alternative method of quantitative assessment of urine protein excretion is measurement of the total protein/creatinine ratio on a spot urine sample. The ratio that is indicative of nephrotic-range proteinuria is greater than 2 mg protein/mg creatinine (200 mg protein/mmol creatinine).

Plasma protein levels are reduced, less than 50 g/l, due to hypoalbuminemia. Albumin level is lower than 30 g/l and may be less than 10 g/l. Electrophoresis shows low albumin and increased alpha 2-globulins and beta-globulins; the level of gamma-globulins depends on the cause of the nephrotic syndrome. For example, IgG levels are markedly reduced in minimal change disease and elevated in systemic lupus erythematosus.

Total cholesterol and LDL cholesterol are elevated, while HDL cholesterol is unchanged or low, particularly HDL2, leading to an increased LDL/HDL cholesterol ratio [14]. Patients with severe hypoalbuminemia have increased triglycerides and VLDL. Apoproteins, apo B, apo CII, and apo CIII, are also elevated. Levels of lipoprotein Lp(a) are elevated and further contribute to an increased risk of cardiovascular and thrombotic complications [15].

Serum sodium is often reduced due in part to hyperlipidemia and in part to dilution from renal retention of water due to hypovolemia and inappropriate antidiuretic hormone secretion. Hyperkalemia may be observed in cases of renal insufficiency. Hypocalcemia is related to hypoalbuminemia, and the level of ionized calcium is usually normal. Hemoglobin levels and hematocrit are increased in patients with plasma volume contraction. Thrombocytosis is common and may reach 5 x 10^8/l or 10^9/l. Fibrinogen and factors V, VII, VIII, and X are increased, whereas antithrombin III, the heparin cofactor, and factors XI and XII are decreased. These abnormalities contribute to a hypercoagulable state.

Patients with nephrotic syndrome should be examined for complications, such as bacterial infections (peritonitis, cellulitis) or thromboembolic events (pulmonary embolism; deep vein, cerebral venous, or renal vein thrombosis) [16, 17]. Despite the increase in extracellular fluid volume, some patients, particularly those with minimal change disease, present with features of decreased circulating volume (abdominal pain, tachycardia, peripheral vasoconstriction, oliguria, and decreased glomerular filtration rate). This is more frequent at onset of nephrotic syndrome or during a relapse [18].

The age at onset of the nephrotic syndrome is predictive of the cause. A nephrotic syndrome occurring during the first 3 months of life (congenital nephrotic syndrome) has most often a genetic cause [19, 20]. The clinical evaluation includes the search for extrarenal symptoms: pseudohermaphroditism, Wilm's tumor observed in children with the Denys-Drash syndrome, ocular malformations (microcoria, abnormal lens with cataracts, and retinal abnormalities) observed in Pierson syndrome, microcephaly, mental retardation, hiatus hernia in Galloway syndrome, nail anomalies, patellar aplasia, or hypoplasia in Nail-Patella syndrome. A nephrotic syndrome starting between 3 and 10 years is most often due to minimal change disease. In older children, other causes are more frequent such as idiopathic FSGS, membranous nephropathy, or IgA nephropathy. In this age group, secondary causes such as systemic lupus erythematosus are also more frequent.

Family history may show another family member affected with nephrotic syndrome or another renal disease and/or parental consanguinity suggesting a genetic form of the illness [21].

Clinical features are predictive of the most common form of nephrotic syndrome, minimal change disease [22, 23]. Minimal change disease represents more than 90% of nephrotic syndrome observed between 1 and 10 years of age. Such diagnosis is likely in patients younger than 10 years, with no syndromic features or family history, in absence of hypertension and hematuria, normal kidney function, and normal blood level of complement. As more than 90% of these patients are steroid responsive, an initial trial of steroid therapy is administered without further investigations.

Some patients with nephrotic syndrome present with additional nephritic features (macroscopic hematuria, hypertension, decreased renal function), suggesting primary (postinfectious glomerulonephritis) or secondary glomerulonephritis. The presence of extrarenal symptoms such as a cutaneous rash, joint pain, and lymphadenopathy favor a secondary form of the illness. Patients with a clinical presentation not suggestive of minimal change should be evaluated with additional investigations according to the clinical features (Table 1).

Table 1 Investigations in a child presenting with nephrotic syndrome

Estimation of urinary protein
Urinalysis to detect hematuria
Blood tests Complete blood count Total protein, albumin Urea, creatinine, estimated glomerular filtration rate Electrolytes: sodium, potassium, ionized calcium Lipids: LDL and HDL cholesterol, triglycerides Coagulation tests (prothrombin time, fibrinogen, ATIII)
Check for causes other than minimal change disease Complement levels (CH50, C3, C4) Antistreptolysin O Antinuclear antibodies, anti-double-stranded DNA; antineutrophil cytoplasmic antibodies Anti-glomerular basement antibodies Anti-PLA2R autoantibodies Serology for hepatitis B and C; Epstein-Barr virus, and human immunodeficiency virus
Renal ultrasound (kidney size, echogenicity)
Genetic screening: extended gene panel, whole exome sequencing
Kidney biopsy (light microscopy, immunofluorescence); electron microscopy (if necessary)

Indications for Genetic Testing

Genetic testing, if available, should be performed in children with primary steroid-resistant nephrotic syndrome, particularly those with familial history of proteinuria and with extrarenal symptoms [24, 25]. Whole exome sequencing technology detect a monogenic disease in 10–30% of cases, the most frequent being *NPHS2*, *WT1*, and *NPHS1* mutations [26]. Identification of pathogenic or likely pathogenic variations in these genes has important clinical implications [27, 28]. For example, immunosuppressive treatment is ineffective and should be withdrawn, and these patients have a low risk of recurrence after renal transplantation. Genetic testing is not recommended for children who respond to steroid therapy with complete remission of proteinuria. Genetic testing is also recommended in patients with congenital or infantile forms of nephrotic syndrome starting before 1 year of age.

Indications for Kidney Biopsy

A biopsy is performed in children presenting with a nephrotic syndrome beyond the age of 12 years, since the proportion of patients with minimal change disease decreases significantly thereafter, and side effects of steroid therapy are more important during puberty. A biopsy is required for children who fail to respond to steroid therapy in order to detect the underlying pathology. In patients with idiopathic steroid-resistant nephrotic syndrome, a kidney biopsy is important before starting treatment with calcineurin inhibitors. A biopsy is indicated when there is suspicion of renal pathology, other than minimal change. This is the case of children with macroscopic hematuria, hypertension, decreased renal function, or low complement level. In these patients, renal biopsy may show membranoproliferative glomerulonephritis, membranous nephropathy, IgA nephropathy, or C3 glomerulopathy (Table 2) [29].

Table 2 Causes of childhood nephrotic syndrome

Idiopathic nephrotic syndrome
Minimal change disease
Primary focal segmental glomerulosclerosis (FSGS)
Genetic diseases
Finnish type congenital nephrotic syndrome (*NPHS1*)
Autosomal recessive steroid-resistant NS (*NPHS2*)
Diffuse mesangial sclerosis (*WT1*, *PLCE1*, *LAMB2*, *NPHS2*)
Denys-Drash syndrome (*WT1*)
Frasier syndrome (*WT1*)
Pierson syndrome (*LAMB2*)
Galloway-Mowat syndrome (*WDR73*, *LAGE3*)
Charcot-Marie-Tooth disease (*INF2*)
Schimke immuno-osseous dysplasia (*SMARCAL1*)
Mitochondrial cytopathies
Alport syndrome (*COL4A5*, *COL4A4*, *COL4A3*)
Fabry disease (*GLA*)
α-1 antitrypsin deficiency (*SERPINA1*)
Infections
Post-streptococcal glomerulonephritis; other bacterial infections (endocarditis, visceral abscess, shunt nephritis); congenital syphilis
Parasitic infections (*Schistosoma mansoni*, *Plasmodium falciparum*, *Toxoplasma gondii*)
Human immunodeficiency virus, cytomegalovirus, polyomavirus, parvovirus B19
Hepatitis B, C
Drugs, toxins
Nonsteroidal anti-inflammatory drugs; gold; heroin; interferon; lithium; mercury; pamidronate; penicillamine; trimethadione
Malignancies
Hodgkin disease, non-Hodgkin lymphoma
Primary glomerulonephritis
Membranous nephropathy
Immune complex membranoproliferative glomerulonephritis
C3 glomerulopathy
IgA nephropathy
Crescentic glomerulonephritis: idiopathic, anti-glomerular basement membrane disease
Secondary glomerulonephritis
Henoch-Schonlein syndrome
Systemic lupus erythematosus
Microscopic polyangiitis; granulomatosis with polyangiitis; Churg-Strauss syndrome
Cryoglobulinemia
Others
Thrombotic microangiopathy; sickle cell disease; diabetes mellitus; amyloidosis; reduced renal mass (oligomeganephronia, renal hypodysplasia); obesity

Cross-References

- ▶ Acute Post-Streptococcal Glomerulonephritis
- ▶ Clinical Aspects of Genetic Forms of Nephrotic Syndrome
- ▶ Congenital Nephrotic Syndrome
- ▶ Genetic Basis of Nephrotic Syndrome
- ▶ Henoch Schönlein Purpura Nephropathy
- ▶ Immunoglobulin A Nephropathy in Children
- ▶ Lupus Nephritis
- ▶ Membranoproliferative Glomerulonephritis and C3 Glomerulopathy in Children
- ▶ Nongenetic Forms of Steroid-Resistant Nephrotic Syndrome
- ▶ Renal Vasculitis
- ▶ Steroid-Sensitive Nephrotic Syndrome

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Genetic Basis of Nephrotic Syndrome

10

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Abstract

Steroid-resistant nephrotic syndrome (SRNS) is a leading cause of end-stage kidney disease in children. Recent findings from genomic studies suggests that 10–30% of all cases of SRNS are monogenic disease where mutation in a single gene is enough to cause disease. More than 60 single gene causes of SRNS have been reported and virtually all these genes localized to the podocyte the glomerular visceral epithelial cell of the kidney hence the term "podocytopathy." The mechanisms by which the mutations in these genes will cause disease is not completely known; however, emerging data showed that some of these genetic defects can disrupt the podocyte F-actin cytoskeleton and the slit diaphragm (SD), signalling at the SD, and also unsettle metabolic activities that are vital for the normal function of the podocyte. Findings from genomic studies is also informing better classification of disease, thus rather than relying on taxonomy that depends on therapy response and nonspecific findings on biopsy, childhood nephrotic syndrome can now be stratified into monogenic NS and immune-based NS. A subset of the latter is readily identifiable as circulating factor disease. This clearly defined disease stratification has implications for approach to therapy and prognostication. Future challenges include developing platforms for the utilization of genetic information in clinical decision process and harnessing multi-omics data and other cutting edge molecular platforms to identify new and specific treatment modalities for nephrotic syndrome.

Keywords

Nephrotic syndrome · Steroid-resistant nephrotic syndrome (SRNS) · Monogenic NS · Genetic mutation · Podocyte

Introduction

Gene discovery efforts aimed at unravelling the causes of Mendelian forms of nephrotic syndrome have resulted in the identification of pathogenic variants in a number of genes that encode proteins crucial for the establishment and maintenance of the glomerular filtration barrier. These discoveries have helped decipher the pathophysiologic mechanisms of the glomerular filtration process by revealing that most of the products of the defective genes are essential for glomerular podocyte function. Before the era of high-throughput genomics, the glomerular basement membrane was thought to be the crucial element of the filtration barrier and its constituents seemed to be good candidates to explain the pathogenesis of familial glomerulopathies. *COL4A* genes implicated in Alport syndrome were discovered almost a decade before the identification of *NPHS1* and *NPHS2* encoding nephrin and podocin, respectively, the key proteins necessary for proper podocyte function.

The discovery of *NPHS1* and later *NPHS2* was the turning point in the glomerulopathy research, and by the year 2000, molecular basis of hereditary nephrotic syndrome started to be resolved. A new category of diseases now named "podocytopathies" was established. Since then, the completion of human genome sequencing and advent of next-generation gene sequencing has greatly accelerated the discovery of new causative disease genes. Subsequently, dozens of genes crucial for podocyte functions were discovered revealing a picture of tremendous genetic heterogeneity for the phenotypically uniform disease. At present, there are 22 entities enlisted in the phenotypic series "nephrotic syndrome" (PS256300) in the Online Mendelian Inheritance in Man (OMIM) Database comprising all known human Mendelian disorders. Another 10 genes are listed in the "focal segmental glomerulosclerosis" (FSGS) phenotypic series (PS603278).

Overall, causative mutations in more than 60 genes have been identified, comprising genes which are selectively or preferentially expressed in the podocyte and encode for protein components of the slit diaphragm (SD), the cytoskeleton, the membrane protein complex linking these structures and intracellular cell signalling. Genetic abnormalities have been identified unexpectedly in cellular structures and pathways hitherto unknown to be involved in the pathophysiology of the disease including mitochondrial energy supply, the nuclear pore complex, and nuclear transcription factors. In recent years, the application of comprehensive genetic testing as the first-line diagnostic tool allowed also for identification of overlapping phenotypes with other hereditary nephropathies such as Dent disease, *DGKE*-associated disease, *TTC21B*-related nephronophthisis, or *PAX2*-related disorders. The list of genes mutated in syndromic and non-syndromic forms of steroid-resistant nephrotic syndrome (SRNS) is constantly evolving and comprises all types of Mendelian inheritance: autosomal recessive, autosomal dominant, X linked, and mitochondrial inheritance (Table 1, Fig. 1).

There is significant variability across the globe with respect to the most common underlying genetic defect in patients with nephrotic syndrome [1]. Although, there are few studies from sub-Saharan Africa and south-eastern Asia, a number of founder mutations have been identified in different geographic regions and populations (Fig. 2). Worldwide, *NPHS2* gene defect is considered the most common cause of hereditary nephrotic syndrome; however, not all ethnic groups share the same frequency of mutations in this gene; for example, in Chinese children and young adults, mutations in *NPHS2* is surpassed by mutations in *COQ8B* gene [2]. Distribution of pathogenic variants reflects geographical isolation, historical migrations, and reproduction in highly consanguineous communities leading to increased frequency of an allele originating from a historical founder effect or a recent bottleneck event in a given population. In general, these refer to a mutation inherited in autosomal recessive manner such as the classic Fin-major and Fin-minor variants in *NPHS1* that is exceptionally prevalent in Finland which was the rationale for the term "Finnish-type nephrotic syndrome" as the name for congenital nephrotic syndrome due to a *NPHS1* defect [3]. The other examples are: c.413G > A (p.Arg138Gln) and c.851C > T (p.Ala284Val) in *NPHS2* prevalent in Western Europe and Hispanic Americans, respectively [4]. The change c.2542G > T (p.Glu848*) in *SMARCAL1* is found frequently in populations of the Central/ Eastern Europe while the c.1339dupG in *COQ8B* reported in Kurdish families is a founder mutation originating from a region in southeast Turkey and c.2492A > C in *NUP107* in East Asians [5–7]. On the other hand, for genes inherited in autosomal dominant manner no founder effect has been reported so far. There are, however, a few recurrent mutation *hotspots* such as *WT1* p.Arg467Trp and c.1447 + 5G > A (previously referred to as p. R394W and IVS9 + 5 G > A respectively), *LMX1B* p.Arg426Gln or *TRPC6* p.Arg895Cys.

Genetic abnormalities, as well as immunological, toxic, infectious, metabolic, hemodynamic, and other mechanisms can alter the podocyte structure and function. Depending on the stage of development and associated environmental factors, podocyte alterations can lead to a wide spectrum of clinical syndromes that should be understood as a continuum and not as separate entities. Indeed, whether the podocytopathies are hereditary or acquired, isolated or syndromic, the molecular perturbations involved in the appearance and progression of renal lesions are closely intertwined. Additional evidence for SRNS/FSGS being a podocytopathy include data from humans and experimental studies that have shown that relative or absolute podocyte depletion or changes in its functional integrity is central to the initiation and progression of the lesion seen in FSGS [8–9]. Wiggins and his group demonstrated the quantitative relationship between podocyte number and evolution of FSGS in a rat model of diphtheria toxin-induced podocyte depletion in which the degree of podocyte loss is regulated. In this model, loss of 30–50% of podocytes seems to be the trigger for progressive kidney damage [9].

Mendelian podocytopathies share the common features of ultrastructural abnormalities of the podocyte foot processes and loss of integrity of

Table 1 Major single gene causes of nephrotic syndrome

Gene	Inheritance	Protein	Function
Slit diaphragm genes			
NPHS1	AR	Nephrin	Signalling between slit diaphragm and actin cytoskeleton
NPHS2	AR	Podocin	Interact with nephrin, link plasma membrane to F-actin cytoskeleton; mechanosensation
CD2AP	AR, AD	CD2 associated protein	Anchors slit diaphragm to actin cytoskeleton
TRPC6	AD	Transient receptor potential channel 6	Regulates calcium homeostasis and chemo/mechanosensation
PLCE1	AR	Phospholipase epsilon1	Modulates slit diaphragm signalling through calcium regulation
FAT1	AR	FAT tumor suppressor homolog 1 (cadherin)	Adhesion molecule signalling through RAC1 and CDC42
KIRREL1	AR	Kin of irregular chiasm-like protein 2 (Nephrin-like protein 1)	Interacts with podocin and nephrin at the slit diaphragm
CRB2	AR	Crumbs homolog 2	Regulator of podocyte polarity and nephrin trafficking
Actin binding and regulator of microtubules			
ACTN4	AD	Actinin alpha 4	Interconnects actin filaments in podocyte foot processes
INF2	AD	Inverted formin 2	Diaphanous-related formin governing cellular phenomenon such as actin cytoskeleton remodelling, cell polarity, morphogenesis and cytokinesis
MYO1E	AR	Myosin 1E	Involved in podocyte actin cytoskeleton organization and motility
ARHGDIA	AR	Rho GDP dissociation inhibitor alpha	Involved in cytoskeletal regulation and cell motility
ARHGAP24	AD	Rho GTPase activating protein 24	Actin regulating gene
Regulator of microtubules and transfer RNA modifiers			
WDR73	AR	WD repeat domain 73	Regulation of the microtubule network and cellular architecture
WDR4	AR	WD repeat domain 4	Regulates universal chemical modification of transfer RNA
OSGEP	AR	O-sialoglycoprotein endopeptidase	Regulates universal chemical modification of transfer RNA
TP53RK	AR	TP53 regulating kinase	Regulates universal chemical modification of transfer RNA
TPRKB	AR	TP53RK binding protein	Regulates universal chemical modification of transfer RNA
GON7	AR	GON7 subunit of KEOPS complex	Regulates universal chemical modification of transfer RNA
YRDC	AR	N6-threonylcarbamoyltransferase domain containing	Regulates universal chemical modification of transfer RNA
LAGE3	X linked	L antigen family member 3	Regulates universal chemical modification of transfer RNA
Transcription factors and nuclear genes			
WT1	AD	Wilms tumor 1 transcription factor	Transcription factor regulating urogenital development
SMARCAL1	AR	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a like 1	Protein involved in DNA remodelling, critical to stability of DNA replication

(continued)

Table 1 (continued)

Gene	Inheritance	Protein	Function
LMX1B	AD	LIM homeobox transcription factor 1 beta	Transcription factor regulating dorso-ventral patterning of limb development and formation of podocytes
Nucleoporins			
NUP85	AR	Nucleoporin 85 (nuclear pore complex protein Nup85)	Nucleoporin supporting the central channel of the nuclear pore complex
NUP93	AR	Nucleoporin 93 (nuclear pore complex protein Nup93)	Nucleoporin making inner the nuclear pore complex
NUP107	AR	Nucleoporin 107 (nuclear pore complex protein Nup107)	Nucleoporin supporting the central channel of the nuclear pore complex
NUP133	AR	Nucleoporin 133 (nuclear pore complex protein Nup133)	Nucleoporin supporting the central channel of the nuclear pore complex
NUP160	AR	Nucleoporin 160 (nuclear pore complex protein Nup160)	Nucleoporin supporting the central channel of the nuclear pore complex
NUP205	AR	Nucleoporin 205 (nuclear pore complex protein Nup205)	Nucleoporin making inner the nuclear pore complex
XPO5	AR	Exportin 5	Mediates transport of proteins and other cargo between nuclear and cytoplasm
Mitochondrial genes			
COQ2	AR	Coenzyme Q2, polyprenyltransferase	Enzyme involved in biosynthesis of CoQ
COQ6	AR	Coenzyme Q6, monooxygenase	Enzyme involved in biosynthesis of CoQ
COQ8B	AR	Coenzyme Q8B	AarF domain-containing kinase involved in CoQ10 biosynthesis
PDSS2	AR	Decaprenyl diphosphate synthase subunit 2	Enzyme involved in biosynthesis of CoQ
MTTL1	Mitochondrial	Transfer RNA leucine 1	Mitochondrial tRNA for leucine (UUR)
Integrin, adhesion, and laminin genes			
LAMB2	AR	Laminin beta 2	Extracellular matrix proteins provide scaffold for assembly of other components of glomerular basement membrane
ITGB4	AR	Integrin subunit beta 4	Receptor for laminins, ITGB4 mediate cell-matrix or cell-cell adhesion, and transduced signals that regulate gene expression and cell growth
ITGA3	AR	Integrin subunit alpha 3	Heterodimerizes with the β1 subunit to form integrin α3β1
LAMB3		Laminin beta 3	Extracellular matrix proteins that provide the basic scaffold for assembly of the other components of the glomerular basement membrane
Phenocopies			
COL4A3 *COL4A4* *COL4A5*	AR, AD, X-linked	Collagen IVA	Components of the glomerular basement membrane
TTC21B	AR	Tetratricopeptide repeat protein 21B	Negatively modulate SHH signal transduction; retrograde intraflagellar transport in cilia. Mutations in *TTC21B* cause of nephronophthisis type 12 (NPHP12)
CLCN5	X-linked	Chloride channel voltage sensitive type 5	Localizes to endosomal membranes; facilitate albumin uptake by proximal tubule. Mutations in *CLCN5* cause of dent disease

(continued)

Table 1 (continued)

Gene	Inheritance	Protein	Function
			and tubular disorders associated with kidney stone
PAX2	AD	Paired box protein type 2	Important transcription factor during kidney development. *PAX2* mutation may cause of optic nerve colobomas and renal hypoplasia

AD autosomal dominant, *AR* autosomal recessive.

the glomerular filtration barrier (GFB) integrity with idiopathic or immune-mediated nephrotic syndrome. By light microscopy, the absence of inflammatory lesions and immune complex deposition distinguishes genetic podocytopathies from other secondary causes of disease. Apart from rare congenital forms, the initial presentation of immune-mediated and hereditary podocytopathies is generally similar. However, family history, resistance to immunosuppressive therapy, progression to end-stage kidney disease, and significantly reduced risk of recurrence after kidney transplantation suggest a genetic origin.

Molecular Mechanisms of Monogenic Nephrotic Syndrome

The podocyte is a component of the trilayer GFB; other components are the specialized fenestrated endothelial layer and the glomerular basement membrane (GBM) **(**Fig. 1**)**. Podocytes are terminally differentiated visceral epithelial cells with a cell body, primary, secondary, and tertiary foot processes that interdigitate with neighboring podocytes to form a highly specialized gap junction, the slit diaphragm. Virtually all the genes that are known to cause SRNS/FSGS localize to different parts of the podocyte and disrupt the structure and function of the podocyte **(**Fig. 1**)**. Below is a description of the mechanisms by which mutations in some of these genes will cause disease.

Slit Diaphragm Genes

The podocyte SD is a specialized intercellular junction that connects neighboring podocytes. It is a complex signalling platform that is vital for the maintenance of the structure and function of the podocyte actin cytoskeleton. Morphologically, most podocytopathies are characterized by loss of podocyte slit diaphragm a clear indication of its central role in maintaining the integrity of the glomerular filtration barrier (GFB). The SD was first recognized as a grey line between neighboring podocytes in 1955 on electron microscopy [10]. Its structure was elucidated by elegant transmission electron microscopy studies and was described for the first time as a “zipper like” structure by Karnovsky and Ryan in 1975 [11]. The zipper was described as being formed by molecules from neighboring podocyte foot process that crosses the intercellular space and overlap to form a dense midline structure [12]. The nature of the zipper or the molecular organization of this structure was not unravelled until more than 20 years later when biallelic pathogenic variants in nephrin gene (*NPHS1*) was identified as a cause of congenital nephrotic syndrome [3]. Nephrin is a transmembrane protein and it formed the backbone of the zipper structure of the SD through homophilic interaction with one another. Since these landmark discoveries, multiple genes causing monogenic SRNS/FSGS have been shown to localize to the SD and are known to be important for maintaining the structure, and dynamic intracellular and extracellular signalling at the SD [13–14]. The full repertoire of the molecular components of the podocyte SD is currently unknown; however, desirable properties of the components of the SD include ability to (i) act as a macromolecular filter, (ii) anchor the filter to the GBM, (iii) connect the SD to actin cytoskeleton through adaptor proteins, and (iv) be part of a signalling complex that integrates and mediates extracellular and intracellular signals regulating the plasticity of podocyte foot processes

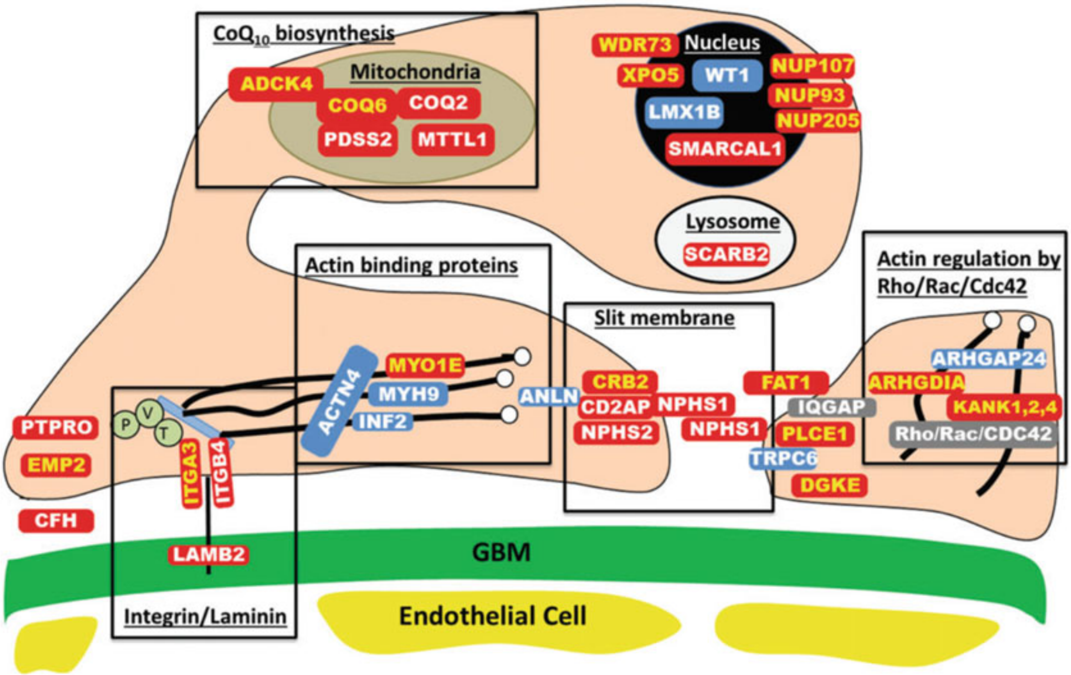
CoQ10 biosynthesis
Mitochondria
ADCK4
COQ6
COQ2
PDSS2
MTTL1
WDR73
Nucleus
XPO5
WT1
NUP107
NUP93
NUP205
LMX1B
SMARCAL1
Lysosome
SCARB2
Actin binding proteins
Actin regulation by Rho/Rac/Cdc42
Slit membrane
MYO1E
MYH9
INF2
ACTN4
ANLN
CRB2
CD2AP
NPHS1
NPHS2
NPHS1
FAT1
IQGAP
PLCE1
TRPC6
DGKE
ARHGAP24
ARHGDIA
KANK1,2,4
Rho/Rac/CDC42
PTPRO
EMP2
CFH
P
V
T
ITGA3
ITGB4
LAMB2
GBM
Integrin/Laminin
Endothelial Cell

Fig. 1 **Proteins involved in single gene causation of steroid-resistant nephrotic syndrome (SRNS).** Identification of single gene (monogenic) causes of SRNS has revealed the visceral glomerular epithelial cell, the podocyte, as central to the pathogenesis. This cartoon shows two neighboring podocyte foot processes, which attach to the GBM via laminin/integrin receptors. Recessive monogenic forms of SRNS are in red, and proteins that if mutated cause dominant forms of SRNS are in blue. These SRNS-related proteins were found to be part of protein–protein interaction complexes that participate in defining the structural components or signalling pathways of podocyte function (black frames). These proteins include: laminin/integrin receptors (focal adhesions), actin-binding proteins, glomerular slit membrane-associated components, actin-regulating small GTPases of the Rho/Rac/Cdc42 family, lysosomal proteins, nuclear transcription factors and proteins involved in coenzyme Q10 (CoQ10) biosynthesis. Proteins encoded by recessive SRNS genes include: ADCK4, AarF domain containing kinase 4; ARHGDIA, rho GDP dissociation inhibitor (GDI) alpha; CD2AP, CD2-associated protein; CFH, complement factor H; COQ2, coenzyme Q2 4-hydroxybenzoate polyprenyltransferase; COQ6, coenzyme Q6 monooxygenase 6; CRB2, Crumbs family member 2; DGKE, diacylglycerol kinase epsilon; EMP2, epithelial membrane protein 2; GBM, glomerular basement membrane; ITGA3, integrin, alpha 3; ITGB4, integrin, beta 4; KANK, KN motif and ankyrin repeat domains 1/2/4; LAMB2, laminin, β2; MTTL1, mitochondrial tRNA leucine 1; MYO1E, Homo sapiens myosin 1e; NPHS1, nephrin; NPHS2, podocin; NUP93, nucleoporin 93 kDa; NUP107, nucleoporin 107 kDa; NUP205, nucleoporin 205 kDA; PDSS2, prenyl (decaprenyl) diphosphate synthase, subunit 2; PLCE1, phospholipase C epsilon 1; PTPRO, protein tyrosine phosphatase, receptor type, O; SCARB2, scavenger receptor class B, member 2; SMARCAL1, SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a-like 1; WDR73, WD repeat domain 73; XPO5, exportin 5. Proteins encoded by dominant SRNS genes are marked in blue: ACTN4, actinin alpha 4; ANLN, anillin; ARHGAP24, rho GTPase-activating protein 24; INF2, inverted formin 2, LMX1B, LIM homeobox transcription factor 1-beta; MYH9, myosin, heavy chain 9; TRPC6, transient receptor potential cation channel, subfamily C, member 6; WT1, Wilms tumor 1; IQGAP, IQ motif containing GTPase activating protein 1; P, paxillin; V, vinculin and T, talin (Adapted with permission from Lovric S, et al. Genetic testing in steroid-resistant nephrotic syndrome: when and how? Nephrol Dial Transplant 2016;31:1802–13. Oxford University Press)

[12]. Defects in multiple genes that are integral to the structure and function of the SD have been reported; they include nephrin (NPHS1), podocin (NPHS2), CD2-associated protein (CD2AP), transient receptor potential channel 6 (TRPC6), and phospholipase C epsilon 1 (PLCE1).

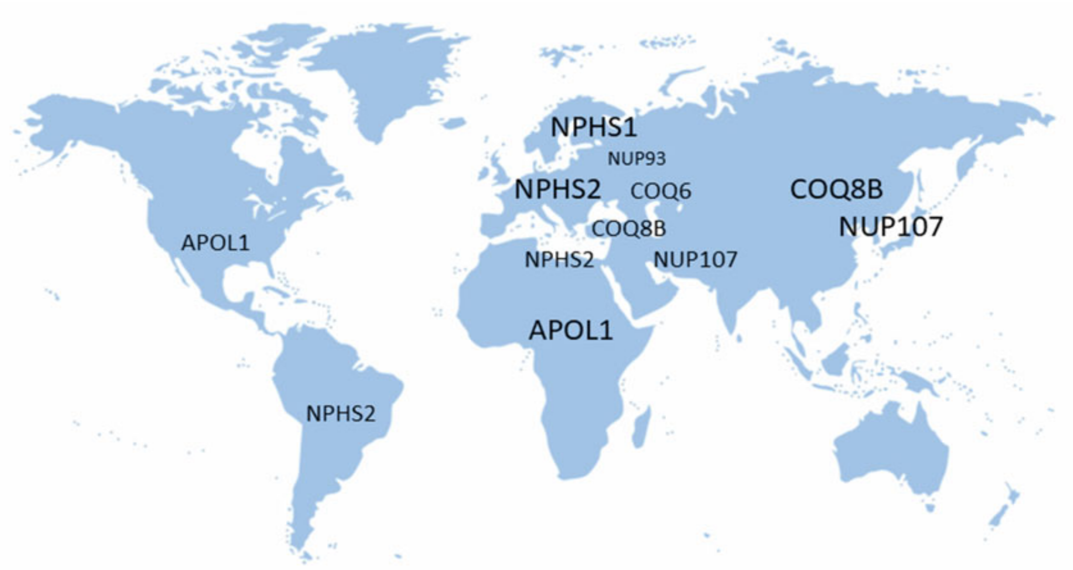

Fig. 2 Geographic distribution of founder mutations in SRNS genes

Nephrin (NPHS1)

Nephrin was identified by positional cloning as a cause of Finnish type congenital nephrotic syndrome in 1998; since then, it has been established that biallelic pathogenic variants in *NPHS1* are found in different populations [3]. Nephrin is a transmembrane protein with a short intracellular domain, a transmembrane domain, and an N-terminal extracellular domain with a proximal type III fibronectin domain and eight IgG like motifs. Homophilic interaction between neighboring nephrin molecules form the zipper-like structure of the slit diaphragm. Nephrin is critical for the formation of podocyte SD, and a SD is absent in both humans and murine models with *NPHS1* defects [12, 14–16]. Nephrin plays a critical role in signalling at the slit diaphragm through phosphorylation of its cytoplasmic tail by tyrosine kinase [15].

Podocin (NPHS2)

The gene *NPHS2* encode for podocin a 383 amino acid protein with a hairpin-like structure with both cytoplasmic, amino, and carboxyl terminal domains [17]. Podocin is an integral membrane protein and it localizes to the insertion site of the podocyte slit diaphragm through a short transmembrane region [18–19]. It is a lipid raft-associated protein belonging to the stomatin family. It also contains a prohibitin homology (PHB) domain, in addition to palmitoylation sites in the hydrophobic region. The palmitoylation sites are required for podocin to bind cholesterol and regulate the activity of associated proteins [20]. Podocin accumulates in oligomeric form in lipid-raft microdomains of the SD plasma membrane where it recruits nephrin thereby augmenting the nephrin-induced activation of the AP-1 transcription factor, and also interacts with CD2AP and TRPC6 [21–24]. The resulting protein complex represents an important link between the SD and podocyte cytoskeleton, and is crucial for structural organization and regulation of filtration function of the SD, mechanosensory signalling, podocyte survival, cell polarity, and cytoskeletal organization [17, 20–24]. Antignac group first reported biallelic pathogenic variants in *NPHS2* as a cause of steroid-resistant nephrotic syndrome [25]. Since then, different reports have shown that mutations in *NPHS2* are the most common cause of autosomal recessive FSGS/SRNS [26–27]. More recently, a founder mutation in *NPHS2* (p.Val260glu) has also been shown to account for a large percentage of SRNS in South Africa [28].

CD2-Associated Protein (CD2AP)

CD2AP is an 80-kilodalton adaptor protein that is critical for stabilizing contacts between T cells and antigen-presenting cells [29]. Mice deficient in CD2AP have been shown to develop nephrotic syndrome and both homozygous and heterozygous *CD2AP* pathogenic variants have been reported in patients with FSGS [29–32]. CD2AP is a scaffold protein that binds nephrin and podocin, and link both to the podocyte actin cytoskeleton [33].

Transient Receptor Potential Channel 6 (TRPC6)

TRPC6 is a nonselective cation channel that is widely expressed but enriched in the podocyte and the SD. Winn et al. reported gain of function mutation in *TRPC6* gene in 2005 as a cause of autosomal dominant FSGS in a New Zealand family [34]. Multiple familial and sporadic cases of FSGS due to *TRPC6* monoallelic pathogenic variants are reported. TRPC6 serves as a chemosensor and mechanosensor that enables podocytes to respond to filtration rate, flow, and pressure changes by remodelling of actin cytoskeleton and foot process contraction with nephrin and podocin to maintain the structure of the SD [18]. TRPC6 activation appears to be mediated by angiotensin II; TRPC6 also interacts with calcium-dependent cystine proteases calpain 1 and 2 to regulate podocyte actin cytoskeleton and motility [16, 35].

Phospholipase C Epsilon 1 (PLCE1)

Biallelic pathogenic variants were reported by Hildebrandt group as a cause of early onset nephrotic syndrome that is characterized by diffuse mesangial sclerosis (DMS) and FSGS on renal biopsy [36, 37]. Interestingly, some individuals in the original report responded to immunomodulation. Follow-up study showed that defects in *PLCE1* might be the most common cause of non-syndromic DMS (diffuse mesangial sclerosis); in addition, another study identified individuals with truncating mutation in *PLCE1* who are asymptomatic suggesting an unusual finding of incomplete penetrance in an autosomal recessive disease [37–38]. PLCε1 is a member of the phospholipase family of enzymes that catalyzes the hydrolysis of polyphosphoinositides to generate second messengers, such as inositol-1,4,5 triphosphate and diacylglycerol [39]. These second messengers are involved in cell growth and differentiation [36–37, 39].

Other genes that localized to and are important for the functions of the SD that have been implicated in the etiology of monogenic NS include *FAT1* (protocadherins), *KIRREL*, and *CRB2* [40–42] (Fig. 1 and Table 1). Studies of hereditary FSGS coupled with staged proteomics and transcriptomic analysis will unravel other key players that are important in maintaining the structure of the podocyte slit diaphragm [43].

Actin Cytoskeleton Genes

The podocyte cytoskeleton is a dynamic network capable of rapid rearrangements; they are finely regulated to maintain the unique octopus-like cell architecture of the podocyte [44–45]. It is composed of microtubules and intermediate vimentin-like filaments in the cell body and primary foot processes and a dense network of microfilaments of actin, myosin, α-actinin, talin, and vinculin in the pedicles [46]. It is bound to the GBM by focal contacts and connected to the SD [47]. As discussed above, the SD is a modified adherens junction that plays an architectural role, but also transmits extracellular signals continuously to the cytoskeleton, underlying the podocyte's plasticity [48].

Alpha Actinin 4 (ACTN4)

ACTN4 encodes the ubiquitous actin-binding protein α-actinin-4 that is highly expressed in podocytes [49]. α-actinin-4 interconnects actin filaments in podocyte foot processes [50]. Most missense monoallelic pathogenic variants in *ACTN4* are located in the residues within or next to the actin-binding site of α-actinin-4 [49, 51]. Some of them have been shown to increase the binding affinity of α-actinin-4 to F-actin, and/or induce α-actinin-4 mislocalization and formation of α-actinin-4 and F-actin aggregates around the nucleus with subsequent impairment of podocyte spreading and motility [49, 51–55].

Knock-in and knockout mouse models and in vitro data suggest both gain-of-function and loss-of-function mechanisms [52].

Inverted Formin Type 2 (INF2)

INF2 encodes for inverted formin 2 a member of the diaphanous-related formin family, with prototypic member being mDia1. Formins are ubiquitous proteins governing various cellular phenomenon such as actin cytoskeleton remodelling, cell polarity, cell morphogenesis, and cytokinesis [56–59]. INF2 contains the formin homology domains FH1/FH2 involved in actin elongation and nucleation, respectively, as well as the diaphanous autoregulatory and inhibitory domains which play a role in INF2 autoinhibition [56–59]. INF2 interacts with the Rho-GTPase Cdc42 and the myelin and lymphocyte protein MAL, both of which are involved in myelination and myelin maintenance [60]. *INF2* variants found in patients with FSGS are found mainly in the inhibitory domain of INF2 encoded by exons 2–4 [56–59]. It has been established in multiple studies that *INF2* monoallelic pathogenic variants are probably the most common cause of autosomal dominant FSGS [56–59]. *INF2* mutations can also cause FSGS associated with the Charcot-Marie-Tooth peripheral neuropathy [60].

Actin-Regulating Protein Rho-GAP 24 (ARHGAP24)

ARHGAP24 encodes the actin-regulating protein Rho-GAP 24, and it is highly expressed in the podocytes [61]. The loss-of-function variant (p. Gln158Arg) causes impaired Rac1-GAP activity of ARHGAP24 [61].

Rho GDP Dissociation Inhibitor α (ARHGDIA)

This gene encodes the Rho GDP dissociation inhibitor α (RhoGDIα) involved in cytoskeletal regulation and cell motility. Biallelic pathogenic variants in *ARHGDIA* are associated with autosomal recessive FSGS and early onset nephrotic syndrome with diffuse mesangial sclerosis and neurological manifestations [62–63]. Interestingly, mouse and zebrafish models can recapitulate the NS phenotype, which can itself be partially rescued by RAC1 inhibitors, paving the way for the therapy of some forms of hereditary NS [62–64].

Myosin 1E (MYO1E)

MYO1E encodes myosin 1E, a member of the ubiquitously expressed class 1 myosins, implicated in podocyte actin cytoskeleton organization and motility. It contains an N-terminal motor-head domain that binds ATP and F-actin, a calmodulin-binding neck domain, and a tail domain with a putative membrane-binding site. It localizes to the podocyte membrane, with enrichment at the lamellipodia tips [65]. MYO1E deficiency in both zebrafish and mouse causes nephrotic syndrome [66–67]. The p. Ala159Pro *MYO1E* variant (affecting a conserved amino-acid residue of the motor-head domain) and the p. Tyr695X* variant (affecting the calmodulin-binding neck domain) were identified in two families with steroid resistance; renal biopsy showed FSGS, focal thickening and disorganization of the GBM, and focal expansion of the mesangial matrix [65]. These lesions were very similar to those of *myo1e* knock-out mice. Myosin 1E is the second myosin identified in the podocyte after the heavy chain of myosin IIA. Non-muscular myosins generate intracellular tension and their interaction with actin could allow the pedicles to counteract the intraluminal hydrostatic pressure, resulting in podocyte plasticity [68–69].

Regulators of Microtubule.

WDR73

WDR73 encodes a WD40-repeated-containing protein that may play a role in the regulation of the microtubule network and cellular architecture, cell cycle, and cell survival. It is located along the microtubules during the interphase and at the spindle poles and astral microtubules during mitosis. Zebrafish embryos inactivated for *wdr73* by morpholinos show a deficit in brain growth resulting from a defect in neural progenitor cell proliferation and differentiation [70–71]. *WDR73* pathogenic variants result in Galloway-Mowat syndrome, a recessive neurorenal disorder with microcephaly and nephrotic syndrome [70–71].

Transcription Factors and Nuclear Genes.

WT1

Wilms tumor 1 gene encodes a transcription factor of the zinc finger family which is the major factor involved in cell differentiation and survival in the developing kidney, urinary tract, and gonads. Its expression persists in podocytes in the adult kidney, and it is required for proper function of the podocyte and the GFB [72]. For that reason, individuals with *WT1* gene defects typically present with a wide range of clinical findings that include glomerulopathy, disorders of testicular development, congenital anomalies of the kidney and urinary tract (CAKUT), nephroblastoma, and gonadoblastoma. While various combinations of findings were designated as certain syndromes (Denys-Drash, Frasier, Meacham or chromosome 11p13 microdeletion), those designations are now recognized as part of a phenotypic continuum and are no longer clinically helpful [73].

LMX1B

LMX1B gene encodes a LIM-homeodomain transcription factor that directs dorsoventral patterning of limb development and formation of the anterior eye and the podocytes [74]. Loss-of-function variants in *LMX1B* gene cause nail-patella syndrome, which presents as dysplastic changes in the nails, knees, elbows, and the presence of iliac horns. Renal involvement occurs in 30–50% of affected individuals with ~15% reaching ESKD in adulthood. Individuals carrying mutations affecting the homeodomain responsible for DNA binding tend to present with nephropathy and minimal skeletal involvement [75]. Variants affecting the p. Arg246 (R246) residue in particular tend to cause FSGS without extrarenal manifestations [75]. The molecular mechanisms of this renal-limited phenotype is unclear; one study showed that the p.Arg246Gln exerted dominant negative and haploinsufficiency effects on the expression of podocyte genes such as *NPHS1, GLEPP1, and WT1* compared with wild-type *LMX1B* [76]. Specifically, the variant mediated downregulation of WT1(-KTS) isoforms in podocytes [76].

SMARCAL1

SMARCAL1 encodes the SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily A-like protein-1 from the SWI2/SNF2 family of ATP-dependent chromatin remodelling proteins. SMARCAL1 uses energy from DNA-activated ATP hydrolysis for DNA remodelling, especially the replication forks, and is critical for stable DNA replication [77]. Biallelic pathogenic variants classically cause multiorgan disorder with progressive glomerulopathy, spondyloepiphyseal dysplasia leading to short stature and peculiar dysmorphic features, defective cellular immunity, bone marrow failure, and cerebral infarcts. However, systemic features are variable with the exception of inevitable progressive kidney disease and end-stage kidney disease [78].

Transfer RNA Modifiers.

Transfer RNAs (tRNAs) are key players in protein synthesis because they decode genetic information organized into messenger RNA codons, translating them into 20 different amino acids. To be active, tRNAs undergo important posttranscriptional modifications. The t6A modification (N6-threonylcarbamoyladenosine) is one of the few universally conserved tRNA modifications implicated in efficiency and fidelity of translation impacting protein synthesis and cellular proteostasis. The t6A modification is catalyzed by YRDC and OSGEP, the latter being part of the multiprotein KEOPS complex. The KEOPS complex contains five subunits LAGE3, OSGEP, TP53RK, TPRKB, and GON7. Recessive pathogenic variants in the autosomal *OSGEP, TP53RK, TPRKB, GON7, YRDC,* or X-linked *LAGE3* genes are associated with Galloway-Mowat syndrome, the phenotypically heterogeneous disorder characterized by neurodevelopmental defects, dysmorphic features and microcephaly with podocytopathy [79]. YRDC and KEOPS subunit defects affect podocyte and neuron proliferation and migration through a decrease in t6A levels and protein synthesis. The fundamental role of the KEOPS complex in cell maintenance is supported by the observation that none of the affected individuals with autosomal recessive pathogenic variants carried two truncating alleles in the mutated gene. *WDR4* encodes a component of the METTL1/WDR4 holoenzyme, involved in the regulation of the highly conserved N7-methylguanosine (m7G) posttranscriptional tRNA modification. *WDR4* biallelic pathogenic variants also cause autosomal recessive forms of Galloway-Mowat disease [80]. This is the second entity of the disease that can be attributed to alteration in a tRNA-modifying enzyme confirming the major role of tRNA modifications in podocyte and neuron physiology.

Nucleoporin Genes

Nuclear pore complex (NPC) proteins (nucleoporins, NUPs) are a class of ~30 proteins that assemble to form NPCs. NPCs serve as the channels that span the nuclear envelope and allow bidirectional nuclear translocation processes. They form a basket-like structure facilitating the transport of proteins, RNAs, and ribonucleoprotein particles between the cytoplasm and interior of the nucleus, hence controlling nucleocytoplasmic transport. In addition, they are also involved in chromatin organization and epigenetic processes [81]. Pathogenic mutations in a number of genes encoding nucleoporins namely: *NUP85, NUP93, NUP107, NUP133, NUP160, NUP205*, and *XPO5* (encoding the NUP-interacting protein exportin 5) were recently described to cause childhood-onset SRNS [7, 82].

Mitochondria Genes

Mitochondriopathies are a diverse group of disorders due to structural, biochemical, or genetic derangements of mitochondria [83]. Renal dysfunction is rare and may result from pathogenic variants encoded by the mitochondrial or nuclear genomes. The mitochondrial genome codes for 13 essential subunits of the mitochondrial respiratory chain, and the 22 transfer RNA (tRNA) and 2 ribosomal RNA (rRNA) genes [84]. Mutations in the mitochondrial sequence encoding tRNA for leucine (UUR) may lead to a wide range of features including MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like syndrome), diabetes mellitus associated with sensorineural hearing loss or an isolated nephropathy. Nuclear genes that can cause syndromic or isolated steroid resistance encode proteins of the coenzyme Q10 pathway [85].

Coenzyme Q10 (COQ10, ubiquinone) is a lipid-soluble component of all cell membranes, vital for transport of electrons from complexes I and II to complex III of the mitochondrial respiratory chain, a potent antioxidant and co-factor of mitochondrial dehydrogenases and pyrimidine synthesis proteins [86]. At least 10 enzymes are required for CoQ10 synthesis, which, when mutated, lead to heterogeneous multisystemic disorders including myopathy, seizures, ataxia, deafness, optic atrophy, and cardiomyopathy. Steroid-resistant nephrotic syndrome may develop in patients with CoQ10 deficiency with or without extrarenal features, due to biallelic pathogenic variants *in COQ2, COQ6, PDSS2,* or *COQ8B* [85]. Since the products of these genes have been identified as being important in the synthesis of CoQ10, supplementation with CoQ10 may be therapeutic.

Integrin, Adhesion, and Laminin Genes.

LAMB2

LAMB2 gene encodes for laminin beta 2, an important component of the GBM. Using homozygosity mapping, *LAMB2* truncating biallelic pathogenic variants were identified in families with Pierson syndrome [87]. The syndrome is characterized by congenital nephrotic syndrome with diffuse mesangial sclerosis and an irregular GBM on kidney biopsy, ocular anomalies mostly microcoria, and developmental delay [87–89]. *LAMB2* defects can cause both syndromic and non-syndromic early onset nephrotic syndrome [90–94]. Laminins are heterotrimeric extracellular matrix proteins that provide the basic scaffold for assembly of other components of the GBM, including type IV collagen, nidogen/entactin, and sulfated proteoglycans [95]. Mice that are deficient in *lamb2* recapitulate the features observed in patients with Pierson syndrome [96]. Interestingly, proteinuria precedes podocyte foot process effacement in these mice suggesting that SD integrity is not sufficient to maintain proper glomerular filtration functions [97]. It should be noted that overexpression of *lamb1* in *lamb2* null mice prevents development of nephrotic syndrome and extend lifespan suggesting that this may be a therapeutic target for nephrotic syndrome due to *LAMB2* mutation [98].

ITGB4 and other integrin genes: Pathogenic variants have been reported in *ITGB4* encoding for the integrin beta 4 subunit in children with syndromic nephrotic syndrome associated with junctional epidermolysis bullosa [99–102]. The mechanisms by which variants in this gene induce glomerular disease are unknown.

Pathogenic variants have also been reported in *LAMB3, ITGA3*, and *CD151* in children with epidermolysis bullosa and nephrotic syndrome [103–105]. The integrin α3 subunit heterodimerizes with the β1 subunit to form integrin α3β1 expressed at high levels in podocytes, where it associates with the tetraspanin, CD151 [104–109] and binds laminins [105–111]. CD151 strongly interacts with integrin α3β1 and is distributed along the GBM [107–110]. Finally, Cd151null mice recapitulate the phenotype seen in humans [112–113].

Genes Associated with Other Renal Phenotypes

Apart from the genes reported above, genes associated with other renal phenotypes have also been reported as genetic causes of SRNS/FSGS. Searching for causative genetic defects in a pedigree of three sisters referred for familial SRNS diagnosed between 8 and 13 years of age and FSGS on biopsy, Gbadegesin's group identified two compound heterozygous variants of *COL4A3* segregating with the disease and showed that 10% of families diagnosed with familial FSGS have pathogenic variants in *COL4A3* or *COL4A4* genes [114]. These findings have been confirmed in other cohorts around the world [115–117]. These data emphasize the need to screen for pathogenic variants in *COL4A3, COL4A4, and COL4A5* in patients with familial FSGS, especially in those with microscopic hematuria or deafness. Pathogenic variants in *COL4A3* and *COL4A4* are responsible for Alport syndrome of autosomal dominant or recessive inheritance, while pathogenic variants in *COL4A5* is responsible for classic X-linked Alport syndrome. The mechanisms by which defects in *COL4A* genes will produce a predominant podocytopathy phenotype is not completely known, but the effect of modifier genes cannot be ruled out. Other proposed mechanisms include altered permselectivity of GBM, abnormal matrix–podocyte interactions, and defective expression or trafficking of GBM matrix components [114]. In addition, abnormal COL4A may not be able to engage integrin on podocyte effectively [118]. Other monogenic kidney disease genes that have been reported to phenocopy FSGS or podocytopathy include mutations in *TTC21B* (a ciliopathy gene) [119], *CLCN5* (cause of Dent disease) [120], and *PAX2* (a congenital anomaly of the kidney and urinary tract: CAKUT gene) [121–122].

Complex Inheritance

A significant fraction of SRNS is due to an underlying monogenic defect; conversely most cases of steroid responsive nephrotic syndrome are likely due to complex interaction between multiple genetic variants (common and rare) and environmental factors, so-called "complex inheritance." The best way to unravel these variants is by genome wide association studies (GWAS). Unfortunately, there are few GWAS focusing exclusively on idiopathic nephrotic syndrome or FSGS. The few published studies have provided exciting data on the genetic architecture of nephrotic syndrome. Example of recent successes are GWAS in people of African ancestry with FSGS and childhood onset steroid sensitive nephrotic syndrome (SSNS).

FSGS in African Americans

Multiple epidemiologic studies in the USA have reported excess of chronic kidney disease and more specifically FSGS in people of African ancestry. Factors associated with this obvious disparity include unknown genetic factors, social status (wealth, education, and employment status), provider factors (implicit bias), and healthcare factors (access to care or mistrust of the healthcare system) [123]. In a landmark study in 2010, the genetic basis for high prevalence of FSGS in African Americans (AA) was established when variants in the gene *apolipoprotein L-1 (APOL1)* were reported to confer 10 times odds of developing FSGS among AA [122]. Two alleles termed "G1 and G2" were reported almost exclusively in people of African ancestry. The G1 allele are two non-synonymous variants that are almost in complete linkage disequilibrium (p.Ser342Gly, p.Ile384Met) and the G2 allele is an in-frame 6-nucleotide deletion (p.Asn388_Tyr389del). *APOL1* encodes a protein that causes lysis of trypanosome the cause of African sleeping sickness. The G1 and the G2 allele provide survival advantage for fatal African sleeping sickness analogous to protection conferred against malaria by sickle cell trait. However, homozygous and compound heterozygous genotype predispose to FSGS and other chronic kidney diseases. Since its original description, the high-risk genotype has been associated with chronic kidney diseases such as HIV-associated nephropathy (HIVAN), sickle cell disease nephropathy (SCDN), and lupus nephritis [124–125]. Limited studies in AA children showed that just like in adults, *APOL1* high-risk genotype is a major risk factor for SRNS/FSGS [126–129]. Although high-risk genotype for *APOL1* is highly penetrant for a polygenic disease risk allele, it should be noted, however, that only about 25% of individuals with high-risk *APOL1* genotype go on to develop CKD, suggesting that there are disease modifiers associated with APOL1-associated nephropathy. The mechanisms by which *APOL1* variants may predispose to FSGS are not unravelled; however, there are suggestions from in vitro and in vivo studies that the variants may predispose to disease by increased expression of APOL1 in the kidney, NLRP3 inflammasome activation, mitochondrial dysfunction, aberrant efflux of intracellular potassium, defective endolysosomal trafficking, and endoplasmic reticulum stress [130–136]. Compounds that can downregulate APOL1 expression are being developed for treatment and prevention of APOL1-associated nephropathy [137].

Childhood Steroid Sensitive Nephrotic Syndrome (SSNS)

SSNS is a relatively rare disease with prevalence of 16 cases per 100,000 children; it is therefore difficult to assemble a large cohort to carry out GWAS study to identify disease risk loci. It is a complex trait that occurs and is determined by the combined effect of different alleles in many genes and interaction with numerous environmental factors such as lifestyle, diet, exposure to certain pathogens, and other factors. Gbadegesin and his group identified variants in *HLA-DQA1* gene as risk locus for SSNS in a modest cohort of South Asian children [138]. Since then, four other studies have confirmed these findings and reported other loci in the HLA region [139–142]. More recent studies have identified variants in *CALHM6* (calcium homeostasis modulator family member 6), *TNFSF15* (tumor necrosis factor superfamily member 15) that are outside the HLA region, but associated with regulation of immune response, as risk loci for SSNS [140–142]. A study from Japan showed that common variants in *NPHS1* (nephrin) are associated with SSNS suggesting that the same gene can cause or contribute to both monogenic disease (congenital nephrotic syndrome) and a more complex, polygenic disease like SSNS [142]. Findings from these association studies emphasize the role of adaptive immunity in the pathogenesis of SSNS. Larger meta-analyses may lead to identification of additional loci and pathways that may be targets for novel and specific therapy.

Implications on Disease Stratification and Diagnosis

Current Classification

Idiopathic nephrotic syndrome, defined by the classic triad of proteinuria, edema, and hypoalbuminemia, is classified according to clinical/observational criteria, which have little known relevance to underlying molecular mechanisms.

There are two main ways of stratification by these means, either by response to steroids or light microscopic appearance of a kidney biopsy. In children, it is more common to classify according to steroid response (steroid sensitive, steroid dependent/frequent relapsing, and steroid resistant) partly because not all patients are biopsied. Findings on biopsy do not always correlate with patterns of steroid responsiveness, though FSGS is usually associated with resistance to steroids and nonresponse to immunomodulators.

Stratification Based on Diagnosis of Single Gene Mutations

The discovery of genetic defects causing nephrotic syndrome has provided molecular insight into the disease. Pathogenic variants associated with nephrotic syndrome have been described in over 60 genes, some causing isolated kidney disease, while others are associated with features in other organ systems (syndromic disease).

Patients with genetic nephrotic syndrome behave distinctly from nongenetic disease, which is assumed to be immune mediated [143–144]. The former rarely if ever responds to immunosuppression, with scattered reports in cases that have partially or rarely completely responded. The latter includes patients with *PLCE1* or *WT1* pathogenic variants. Several series are reported where a small proportion of patients with monogenic nephrotic syndrome respond partially or rarely completely, but it can be difficult to distinguish those responses from the natural fluctuations in disease course, or to be confident that all reported variants are pathogenic [143]. Larger series where patients have been comprehensively tested suggest that the actual response rate to immunosuppression in genetic nephrotic syndrome is negligible [143–145]. This suggests that genetic nephrotic syndrome is a different mechanistic group from nongenetic illness and is further backed up by differences in outcome posttransplantation.

In SRNS, most patients reach end-stage kidney disease and require transplantation. In this group, approximately 30–40% will get rapid posttransplant allograft recurrence of the disease, suggesting the presence of a pathogenic circulating factor in the recipient. Focused clinical and genetic screening of SRNS patients has clarified that “circulating factor disease” is a distinct entity from genetic NS, and the former can be better predicted using certain phenotypic criteria alongside genetic testing. The best clinical predictor to date is of patients with secondary steroid resistance (initial steroid sensitivity with late resistance) where patients who initially completely respond to standard steroid therapy become steroid resistant during the course of their disease. Up to 90% of these patients will suffer disease recurrence posttransplantation [146–147].

Comprehensive genetic screening is widely available and allows clinicians the first level of stratification at presentation, where a rapid diagnosis allows decisions on the level of immunosuppression, as well as prognostic ability to predict posttransplant recurrence, and provide information on how fast specific genetic mutations will progress.

Immune-Based Nephrotic Syndrome

In non-monogenic nephrotic syndrome, there is still evidence that underlying genetic architecture plays a significant part in driving the disease process. For example, there are at least 22 reported single nucleotide variants in different genes, which are present in significantly higher frequency among cohorts of patients with idiopathic nephrotic syndrome than in healthy controls (complex inheritance). These polygenic risk variants are genetic variants that individually are insufficient to cause a disease but create a high-risk disease genotype when combined with other genetic and environmental factors. These genetic factors may represent genetic modifiers that render a patient susceptible to (or protected from) developing nephrotic syndrome. Most consistent among these are the HLA genes, particularly *HLA-DQA1, HLA-DRB1, and HLA-DQB1*, mainly derived from GWAS approaches, and implying a close link with the immune system [138–142]. This begins to lead to important clues towards genetic modifiers that could be used for further stratification approaches. In other diseases, such as diabetes, GWAS has led to the identification of genetic loci that associate with disease and disease subtypes. This has permitted the development of "genetic risk scores" by assigning weighted scores to each genetic locus, and by combining the score for each individual patient a "risk" of having for example type 1 diabetes [148]. Similar scores may be derived for nephrotic syndrome, that align with pattern of steroid response, or circulating factor disease.

Circulating Factor Disease

The other unique aspect of nephrotic syndrome biology that can be exploited is the observation that a proportion of cases are caused by a circulating factor (or factors) that should in theory be detectable in a blood sample. The target of such factor(s) is the podocyte, so a biomarker based on in vitro assays of human podocytes exposed to patient plasma samples has been a goal of scientific efforts. The readouts include molecular pathways within the podocyte that are activated by disease relapse plasma, with specificity, that distinguishes the disease from off-target effects of human plasma, and that therefore will distinguish circulating factor disease from genetic and other causes of nephrotic syndrome. To date, there is no clear evidence of any single gene mutation being a cause of circulating factor disease. Evidence for this includes the lack of patients with familial recurrence of nephrotic syndrome post-transplantation. Additionally, the premise that circulating factor disease is separate from monogenic disease is further supported by exome sequencing findings from UK cohort of patients with SRNS**.** None of the 25 patients who were initially steroid sensitive had monogenic disease, but 4 of 5 patients had post-transplant recurrence, the archetypal circulating factor disease [143]. This study also identified 108 patients who screened negative for monogenic disease but presented with primary steroid resistance. This group had a high rate of progression to ESKD and a greater than 50% rate of recurrence post-transplantation, suggesting that at least half of those patients have a circulating factor. The remainder could have a yet undiscovered monogenic disease or another unsuspected mechanism of disease.

Other Means of Stratification Based on Molecular Signatures

As our ability to generate and process "big data" from patients with nephrotic syndrome grows, the opportunities for molecular classification has increased [149]. Our biological datasets have comprised of histology, urinary sediment, and plasma chemistry. Genetic sequencing information is now at the vanguard of new perspectives of the disease, utilizing omics technologies. Insights are being made with, e.g., proteomics analysis of biopsy tissue; RNA sequencing of lymphocytes; epigenetic profiling; and metabolomics of patient plasma. The opportunity now is to integrate these diverse datasets to develop molecular signatures for subsets of disease that correlate with the underlying mechanistic drivers. This will lead ultimately to our ability to target both new and existing therapeutic drugs to the patients most likely to benefit, based on molecular pathways involved.

Diagnostic Implications

The discovery of more than 60 genes that can cause SRNS when mutated, alongside the advent of next generation sequencing technology means that clinical genetic screening for patients is now a feasible in clinical practice [150]. Various laboratories have been set up for specific gene panel tests, based on NGS technology and covering some or all of the known genes. Results are available within weeks; these advances have implications for clinicians and patients. Firstly, it is important for clinicians to become familiar with interpretation of results from these reports. Results can be reported using a variety of templates, containing different amounts, levels, and layouts of information. There are multiple levels of uncertainty surrounding the result itself (e.g., testing error) and what it actually means (e.g., variant significance). Guidelines for genetic test reporting are available from the American College of Medical Genetics and Genomics (ACMG), European Society of Human Genetics (ESHG), and Association for Clinical Genetic Science (ACGS) [151–153].

Clear communication of the uncertainties inherent in reports to parents and patients is crucial, and the support of clinical geneticists is often required. Finally, the availability of a rapid genetic test at the time of initial presentation could mean that a diagnostic biopsy can be delayed or avoided, depending on the speed of that result.

The question on who to test is pertinent, given that gene panel testing is relatively inexpensive, and that at least 10–30% of incident patients with SRNS are found to have defects in a single gene responsible for their disease [143, 154]. The cost-benefit ratio strongly advocates routine genetic testing for all children presenting with SRNS. To date, patients with secondary (late) SRNS or SSNS have not been found to have any of the known genetic variants associated with their disease, so routine testing in those groups is not advocated [155]. Genetic testing in SRNS patients presenting in adulthood is less common, and data is scarce. However, recent studies have suggested that in patients with sporadic SRNS with no family history or syndromic features, there is 11–12% incidence of monogenic disease [156–157]. The majority of these present in younger adults, and pathogenic variants are predominantly in *COL4A* genes, illustrating the phenotypic variability of mutations and suggesting that focused gene testing is an alternative way of picking up the majority of genetic diagnoses in the adult population.

Future Directions

New genes continue to be discovered that cause SRNS when mutated, though it is probably safe to say that the majority of disease-causing genes in terms of patient numbers have been identified, at least in the major population groups. The remaining discoveries will mostly be "private" mutations, meaning the genes/mutations will only affect a handful of patients and pedigrees.

Genetic testing now needs to be widespread, rapid, and routine, so that it is embedded into the clinical pathway, and granular understanding of whether specific mutations may respond to existing immunotherapies is required. Collaborative analysis of genotype/phenotype across the world through registries will enable stratification of SRNS, at the level of genetic versus immune disease, and at the level of clinical outcomes for specific genes and variants. A major challenge is the development of specific therapies, based on molecular understanding from study of the newly discovered proteins and pathways. Examples include inhibitors of channels, for example, TRPC6 and TRPC5, or chaperones for misfolding and hence mistrafficked proteins [158–159]. Another approach is by gene therapy technologies, which have been applied successfully for monogenic diseases in other organ systems, for example hemophilia A or spinal muscular atrophy. The major challenge is targeting of viral vectors to the specific affected cell – the podocyte.

In summary, the discovery of genetic causes for SRNS over the last 20 years has transformed not only our understanding of the biology of the glomerular filtration barrier but also transformed patient diagnostic pathways and care.

Cross-References

▶ Clinical Aspects of Genetic Forms of Nephrotic Syndrome
▶ Congenital Nephrotic Syndrome
▶ Inherited Diseases of the Glomerular Basement Membrane

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Congenital Nephrotic Syndrome

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Abstract

Children with congenital nephrotic syndrome (CNS) present within the first 3 months of life. Primary CNS is typically caused by mutations in genes encoding for components of the glomerular filtration barrier. The classical form is the Finnish type of CNS (CNF, NPHS1) caused by mutations in *NPHS1* (nephrin) gene, and it typically causes heavy proteinuria in the newborn period. Pathogenic variants in other genes namely *NPHS2* (podocin), *WT1* (Wilms tumor suppressor 1), *LAMB2* (laminin β2), and *PLCE1* (phospholipase C epsilon 1, NPHS3) can also cause CNS, which have more variable clinical manifestations including a wider age range at disease onset. Mutations in these five genes together account for >80% of CNS patients. Besides genetic causes, CNS can be secondary to other diseases like congenital infections or immune disorders. The diagnosis of CNS and its underlying etiology is based on several features including clinical presentation, family history, laboratory findings, genetic testing, and histology. While medical management is possible for certain forms of secondary CNS, kidney transplantation is the only curative treatment for most patients with genetic CNS. In unstable patients with refractory nephrotic states, bilateral nephrectomy and dialysis followed by transplantation may be considered after they attain body weights of 7–10 kg. In stable patients, nephrectomy is not needed, and renal replacement therapy is commenced when they reach end-stage kidney disease (ESKD). Transplantation should be performed when the patient is no longer nephrotic. The outcome of transplantation in CNS patients is satisfactory, and comparable to other pediatric kidney patients.

Keywords

Congenital nephrosis · Nephrin · Podocin · *WT1* · *LAMB2* · Kidney transplantation

Synonyms

CNF: Congenital nephrotic syndrome of the Finnish type

Congenital nephrotic syndrome (CNS) is defined by the presence of heavy proteinuria, hypoalbuminemia and severe edema, manifesting in utero or in the first 3 months of life. The etiology of CNS is broadly classified into primary causes mostly due to genetic defects, and secondary causes due to systemic disease.

Primary Nephrotic Syndrome

Nephrin (*NPHS1*) and CNS of the Finnish Type (CNF)

The *NPHS1* gene, which encodes the cell adhesion protein called nephrin, was first discovered in 1998 to be the cause of CNS of the Finnish type (CNF) [1]. CNF is a severe form of CNS typically seen in Finnish newborns [2] with an incidence of 1:8200 live births [1, 2]. Since the discovery of nephrin, both CNF and NPHS1 (nephrotic syndrome type 1) were used interchangeably as abbreviations for this disorder. Nephrin localizes to the slit diaphragm and plays an important role in the glomerular filtration process. Two important founder *NPHS1* mutations, Fin-major (c.121delCT; p.L41fs) and Fin-minor (c.3325C_T; p.R1109X), were observed in over 90% of patients with CNF [1]. Both mutations result in stop codons and truncated proteins not expressed in the podocyte slit diaphragm. These mutations are rare outside Finland.

More than 200 *NPHS1* mutations have been reported in non-Finnish CNS patients. They account for the majority (40–80%) of patients with CNS worldwide [3–6]. Most mutations found in non-Finnish patients are missense mutations, though deletions, insertions, nonsense, and splice site changes spanning across the whole gene occur too. Many missense *NPHS1* mutations cause misfolding of the protein and defective intracellular transport resulting in retention of the mutant protein in the endoplasmic reticulum, and absence of nephrin in the slit diaphragm [7]. This may explain why missense *NPHS1* mutations, though considered as "mild," can cause heavy proteinuria and severe CNS [8–10]. Nephrotic syndrome is diagnosed within the first week of life in 82% of the infants with *NPHS1* defects and within 2 months in the rest of the cases. In a European study, all patients with *NPHS1* mutations manifested within the first 90 days [3].

There is no renal histology pathognomonic for *NPHS1* but the most characteristic histological findings are expansion of the glomerular mesangium and irregular microcystic dilations in the proximal tubules [8, 9]. Electron microscopic examination show effacement of the podocyte foot processes and absent podocyte slit diaphragm [10]. Within the first 1–2 years, glomerular sclerosis, interstitial fibrosis, and inflammatory infiltrates increase [9]. Infants with *NPHS1* genetic defects do not have extrarenal malformations [8]. However, minor functional disorders in the central nervous system and heart are common during the nephrotic stage. Most children have hypotonia. Mild atrophic brain changes were seen in a third of the infants with *NPHS1* defects and dystonic cerebral palsy in 8% of Finnish patients. The pathogenesis of these is not known. Minor cardiac findings such as hypertrophy and mild pulmonary stenosis have also been reported in patients with CNF [8]. A subset of patients with certain *NPHS1* variants has been reported to have milder clinical course [4, 11]. In addition, some patients respond to therapy with angiotensin-converting enzyme inhibitors (ACEi) and indomethacin with residual mild proteinuria [8].

Podocin (*NPHS2*)

The *NPHS2* gene, encoding for the protein podocin, is a common cause of steroid-resistant nephrotic syndrome in children and adults. It is the second most common gene (17–39%) implicated in CNS cases after *NPHS1* [12–15]. Patients with *NPHS2* pathogenic variants present later compared to variants in *NPHS1* [3]. Among all European patients with CNS presenting in the first week of life, only 11% had *NPHS2* defects while 74% had *NPHS1* variants. Among those presenting with nephrotic syndrome after 1-month of age, *NPHS2* accounted for 37%, whereas *NPHS1* variants were present in 16% [4]. The prevalence of *NPHS2* mutations differs in different ethnic groups. None of the 25 Turkish patients with CNS carried *NPHS2* mutations, while 54% of central European infants with CNS carried *NPHS2* pathogenic variants [3]. Studies in Asia suggest that *NPHS2* mutations are relatively rare in patients with CNS (0–8%) [13, 16, 17].

The severity of proteinuria and clinical findings are more variable than in patients with *NPHS1* defects. Although most patients with *NPHS2* mutations presenting in later childhood have focal segmental glomerulosclerosis (FSGS), CNS patients with *NPHS2* mutations often show minimal histological changes, and <50% have FSGS [4]. Progression to ESKD is less rapid for CNS patients with *NPHS2* mutations (mean 6.6 years) compared to *NPHS1* mutations (mean 4.6 years) [3]. As *NPHS2* is only expressed in the podocytes, extrarenal manifestations are unlikely.

Wilms Tumor Suppressor 1 (*WT1*)

Wilms tumor suppressor gene (*WT1*) encodes for a nuclear WT1 protein, a transcription factor that plays a crucial role in the embryonic development of the kidney and genitalia. Being an autosomal dominant gene, heterozygous *WT1* genetic defects account for 2–13% of cases with CNS [6, 18]. Missense mutations in exons 8 or 9 impair the DNA-binding capacity of the WT1 protein and can result in Denys-Drash syndrome that is characterized by early nephrotic syndrome with diffuse mesangial sclerosis, male pseudohermaphroditism, Wilms tumor, and gonadoblastoma. Truncating genetic variants are associated with highest risk of Wilms tumor, while missense variants affecting the DNA-binding sites are associated with early disease onset and rapid progression to ESKD. Male pseudohermaphroditism with the highest risk of gonadal malignancy occurs exclusively in patients with missense variants at the DNA-binding sites. Unlike syndromic cases which can occur in both male and female phenotypic patients, the non-syndromic isolated nephrotic syndrome that manifests at varying ages, occur exclusively in genotypic and phenotypic females [19].

Patients with *WT1* mutations have varying degrees of proteinuria and are less symptomatic at disease onset with less marked edema and hypoalbuminemia compared to other genetic diagnosis. While they tend to have larger placentas compared to normal infants, their placenta (placental/fetal weight 18%) are significantly smaller compared to patients with CNF (placental/fetal weight >25%). Patients with Denys-Drash syndrome are likely to have higher serum creatinine at onset compared to CNF [18]. The characteristic and commonest glomerular lesion is diffuse mesangial sclerosis, although FSGS and minimal change disease can also occur [19]. Urogenital abnormalities are common.

Because of the significant and early risks of Wilms tumor (55–74%) [20, 21], the management of patients with *WT1* variants is different from other CNS patients. Prophylactic bilateral nephrectomy after onset of ESKD is recommended. A pediatric endocrinologist should be involved in the multidisciplinary care, and prophylactic bilateral gonadectomy should be considered.

Laminin β2 (*LAMB2*)

LAMB2 encodes the laminin β2-chain that is expressed in the glomerular basement membranes (GBM), ocular structures, and neuromuscular synapses. *LAMB2* genetic variants account for 1.7–5% of patients with CNS [3, 6, 22]. Majority of *LAMB2* mutations are truncating with loss of function and can occur along the entire gene. Patients with *LAMB2* genetic defects classically present with Pierson syndrome which is characterized by early onset nephrotic syndrome associated with distinct ocular malformations [23, 24]. Rapid progression to ESKD may occur during the first few weeks or months of life.

The characteristic ocular feature is microcoria manifesting as fixed extreme narrowing of the pupils. Other ocular manifestations include abnormalities of the lens, cornea, and retina; patients often develop blindness. Patients who survive the newborn period may develop neurological deficits, including severe hypotonia and psychomotor retardation. On the other hand, missense *LAMB2* variants and small in-frame deletions cause a milder phenotype (e.g., milder ocular changes, slower ESKD progression) [24]. *LAMB2* variants rarely cause isolated nephrotic syndrome [3, 24].

In a large cohort of 51 patients from 39 unrelated families, 90% of patients with *LAMB2* mutations presented in the first 3-months of life [25]. Diffuse mesangial sclerosis was the major histological finding, though some had FSGS. Microcoria or other ocular abnormalities were detected in most patients. Microcephaly or neurodevelopmental deficits were present in 10% and 50% patients, respectively [25].

Phospholipase C Epsilon 1 (PLCε1)

Mutations in the phospholipase C epsilon 1 (*PLCE1*) gene cause an autosomal recessive form of early-onset nephrotic syndrome [26]. PLCε1 is a phospholipase enzyme and may be involved in slit diaphragm signaling and the function of the glomerular filter. The absence of PLCe1 may halt glomerular development leading to lesions resembling diffuse mesangial sclerosis. Mutations in *PLCE1* were originally reported in 14 patients from 7 families with isolated SRNS manifesting between 2 months to 9 years [26]. Two patients with truncating *PLCE1* genetic variants achieved full and sustained remission with cyclosporine A or steroids. Truncating *PLCE1* mutations were found in 29% of a worldwide cohort of children with “idiopathic” diffuse mesangial sclerosis manifesting between 1 and 36 months [27]. Among CNS, *PLCE1* pathogenic variants accounted for 4.4% cases [3]. While diffuse mesangial sclerosis is the chief histology, FSGS may also be found. Incomplete penetrance occurs, suggesting that other factors might modify the effects of *PLCE1* variations [6]. No extrarenal manifestations are noted in patients with *PLCE1* genetic variants.

Other Genes

Galloway-Mowat syndrome, an autosomal recessive condition, is characterized by nephrotic syndrome, severe neurological abnormalities and microcephaly at birth. Brain abnormalities include neuronal-migration abnormalities such as agyria or microgyria, which lead to severe psychomotor developmental delay and seizures. The patients may also present dysmorphic features such as hypertelorism, ear abnormalities, and micrognathia. Most die in early childhood. Truncating variants in the *WDR73* gene were first described as the cause of the disorder [28]. More recently, pathogenic variants in *OSGEP*, *LAGE3*, *TP53RK*, and *TPRKB* genes have also been identified as monogenic causes of the disease. These genes encode the four subunits of KEOPS complex, which plays an important role in the brain and renal development [29].

Mitochondrial disorders can cause glomerulopathy. These are broadly divided into two major types: defects in a nuclear gene, namely *COQ2*, *PDSS2*, *COQ6*, *ADCK4*, or *PDSS1,* encoding proteins in coenzyme Q10 (CoQ10) biosynthesis pathway (autosomal recessive inheritance), and those caused by mitochondrial DNA 3243 A > G mutation in the tRNALeu (UUR) gene (maternal inheritance). These patients show FSGS that may be collapsing or associated with crescents. Patients with CoQ10 biosynthetic defects may have a glomerulopathy occurring either in isolation or as part of a multisystemic disease. The coexistence of neuromuscular symptoms with renal defects raises the suspicion of a mitochondrial disorder. Age of onset varies and may be within the neonatal period [30]. The first symptoms of mitochondrial disorder develop within the first few weeks of life in about one-third of patients. In CoQ10 biosynthetic defects, treatment with high doses of CoQ10 at 30–50 mg/kg/day can reduce proteinuria, improve symptoms of nephrotic syndrome, and slow progression of the disease [31, 32].

SGPL1 encodes sphingosine-1-phosphate lyase 1 that catalyzes the final degradation step of sphingolipid that is involved in angiogenesis, vascular maturation, and immunity. Biallelic *SGPL1* pathogenic variants can cause significant glomerulopathy. Out of the 30 patients reported to have *SGPL1* pathogenic variants, 8 presented with CNS, while 4 had fetal demise [33–35]. The most significant extrarenal manifestation is primary adrenal insufficiency. Other extrarenal manifestations include ichthyosis, immunodeficiency, and neurological abnormalities including sensorineural hearing loss. A few other rare genetic causes of primary CNS have been described, such as ARHGDIA [36], MAGI2 [37], CRB2 [38], Lowe syndrome [39], and congenital disorders of glycosylation [40].

Secondary Nephrotic Syndromes

CNS may be secondary to some systemic diseases, including congenital or neonatal infections, and immune disorders.

Infections

Congenital syphilis has been known to cause CNS. The prevalence of CNS was 2.4% among neonates and infants with congenital syphilis in Thailand [41]. Neonates with syphilis usually present with nephrotic syndrome at 2–3 months of age, although presentation in the neonatal period may occur. Membranous nephropathy is a common biopsy finding. Antimicrobial therapy, usually penicillin, is curative if irreversible renal lesions have not developed [42].

Congenital cytomegalovirus infection, associated with certain strains of the virus, may be associated with CNS [43]. Neonatal CMV infections have also been reported to cause CNS [44]. Renal histology shows diffuse mesangial sclerosis, and patient respond satisfactorily to therapy with ganciclovir.

Toxoplasmosis has been associated with CNS, together with neurologic or ocular features [45]. In one report, a 3-month-old girl with CNS and toxoplasmosis responded well to spiramycin therapy [46]. Congenital rubella can cause CNS due to membranous glomerulonephritis [44]. While acquired immunodeficiency syndrome caused by the human immunodeficiency virus may be associated with nephropathy, including nephrotic syndrome that presents in older children, no patients with CNS caused by this infection has been reported [44, 47]. Hepatitis B virus commonly causes membranous nephropathy in children and adults, but has not been reported to cause CNS. Other infections that might be rarely associated with CNS include measles, pertussis, herpes simplex and herpes zoster.

Immune Disorders

Although systemic lupus erythematosus is rarely diagnosed before 5 years of age, an infantile form of the disease has been reported. Nephrotic syndrome was the major clinical finding in five infants aged 6 weeks to 6 months with systemic lupus erythematosus [48]. These patients had elevated antinuclear antibody titers, hypocomplementemia, and diffuse proliferative glomerulonephritis. Response to the immunosuppressive therapy was unsatisfactory.

Nephrotic syndrome due to membranous nephropathy has been observed antenatally in infants from mothers with mutations in the metallomembrane endopeptidase gene, which encodes the neutral endopeptidase (NEP) expressed on podocytes. During pregnancy, the fetal NEP protein induces a maternal alloimmune response, resulting in the production of maternal antibodies to fetal NEP protein. This in turn leads to glomerular subepithelial immune deposits in the fetus and neonate [49]. The infant presents with nephrotic syndrome, but with time the proteinuria decreases as the maternal antibodies and immune deposits decline. However, persistent proteinuria and chronic renal insufficiency may be observed due to nephron loss and renal scarring.

Diagnosis

Clinical Findings

A thorough evaluation of a newborn baby or small infant with suspected CNS is important (Table 1). Family history may reveal previous fetal or infant deaths, parental consanguinity, autoimmune diseases, or early-onset neurological or renal diseases. Antenatal history of hydrops fetalis, growth retardation, or maternal infections may be present. Most Finnish children with *NPHS1* mutations are born premature with mean birth weight of 2600 g. Placentas may be larger than normal. Amniotic fluid is often meconium stained, although most neonates do not have pulmonary problems.

Classical features of CNS include generalized edema, abdominal distension, and ascites. The degree of proteinuria varies and hence clinical signs of CNS may not be evident during the first weeks of life. Blood pressure levels may be low due to low oncotic pressure related to hypoproteinemia, or elevated if kidney impairment has developed. In severe forms of CNS, urinary protein >20 g/l, and serum albumin level <10 g/l is noted right after birth. The severity of proteinuria may only manifest after partial correction of hypoalbuminemia with albumin infusions. Microscopic hematuria or leukocyturia may be present. While oliguria is common, blood levels of creatinine and urea levels are variable. Most patients with *NPHS1* mutations have normal renal function in the first few months, whereas rapid deterioration of kidney function may occur in other genetic forms. Profound hyperlipidemia is often present in severe CNS. Complications associated with CNS include sepsis, thrombosis, and hypothyroidism.

Physical investigations should look for extrarenal manifestations that suggest genetic causes, including microcephaly (Galloway-Mowat

Table 1 Evaluation of an infant with suspected CNS

	Diagnosis	Primary form (genetic)	Secondary causes
History	Prenatal ultrasonography: kidney size, echogenicity Gestation (<37 weeks) Birth weight, height Large placenta Respiratory problems; meconium-stained amniotic fluid	Affected siblings Previous infant deaths, miscarriage in family Consanguinity (mostly recessive inheritance)	Congenital infections (fever with rash, cat exposure, serology, PCR) Maternal mutations in neutral endopeptidase (NEP)
Examination	Edema, ascites Cardiovascular exam Systemic infections Urine output	Extrarenal features (dysmorphic features; skeletal, neurological, cardiac, visual or hearing defects; genitalia)	Extrarenal features (rash, hepatosplenomegaly, cataract; cardiac or neurological abnormalities)
Urine, blood investigations	Urine protein to creatinine ratio, urinalysis Blood urea, creatinine, electrolytes, albumin, glucose, thyroxine, thyroid stimulating hormone, coagulation status, lipids	Gene panel: *NPHS1, WT1, NPHS2, LAMB2, PLCE1* Extended gene panel, exome sequencing (if needed) Karyotype (*WT1*) Adrenal function (*SGPL1*) Metabolic workup (mitochondriopathies)	Maternal serology Patient serology; blood DNA/RNA (syphilis, toxoplasmosis, CMV, rubella, measles, pertussis) Immunology: lupus serology; complement C3, C4; anti-NEP antibodies
Imaging (where appropriate)	Abdominal ultrasound (kidney size, echogenicity) Chest X-ray Doppler studies (if thrombosis suspected) Echocardiography	Brain imaging Skeletal imaging Pelvic ultrasound Echocardiography (*NPHS1, NPHS2,* mitochondrial disorders)	Brain imaging Skeletal imaging Echocardiography

syndrome), neurological disorders (Galloway-Mowat syndrome, *LAMB2*, *SGPL1,* mitochondriopathies), abnormalities of the genitourinary tracts (*WT1*), eye abnormalities (*LAMB2, SGPL1,* mitochondriopathies), sensorineural hearing loss (mitochondriopathies, *SGPL1*), and skeletal disorders (nail-patella syndrome) [6]. In low-resource settings where genetic testing is not available, clinicians may consider pelvic ultrasound scans and chromosomal karyotyping in infants with complete female or ambiguous genitalia to look for male pseudohermaphroditism (Denys-Drash syndrome). Karyotyping is also useful in patients with confirmed *WT1* mutation to assess gonadal malignancy risks. Cardiac structural defects are rare, but ventricular hypertrophy may be present. Patients with *NPHS1*, *NPHS2*, and *PLCE1* genetic defects do not have extrarenal malformations. Clinicians should assess for signs of congenital infections like rash or cutaneous scars, cardiac anomalies, hepatosplenomegaly or jaundice, eye defects, and neurological or skeletal abnormalities. If congenital infections are suspected, a search for specific antibodies, or bacterial or viral DNA/RNA by polymerase chain reaction is required (Table 1).

Kidney Biopsy

A variety of histopathological changes can be found in CNS, such as minimal change disease with or without mesangial expansion, FSGS, and diffuse mesangial sclerosis. Nonspecific tubulointerstitial changes such as tubular dilatation, interstitial fibrosis, and inflammation may be seen, as in many proteinuric diseases. Renal histological changes do not correlate with genotypes. If the genetic diagnosis is apparent based on clinical features (ambiguous genitalia suggesting *WT1,* or eye anomalies suggesting *LAMB2* defects), or if genetic testing has been or will be performed promptly, then the kidney biopsy may not be necessary at initial diagnosis. The biopsy may help provide information on the presence and extent of glomerular sclerosis and interstitial fibrosis, and might impact treatment strategies. On the other hand, in the interpretation of sclerosis or fibrosis in renal biopsies, clinicians should be wary of errors due to focal sampling.

Genetic Testing

Genetic testing is preferred for the definitive diagnosis of primary CNS. Analysis of *NPHS1*, *NPHS2, WT1*, *LAMB2*, or *PLCE1* mutations uncovers the genetic etiology in >80% of cases, and is warranted in all patients [6, 50]. If this analysis is negative, a more comprehensive screening using either an expanded gene panel (for other genes implicated in nephrotic syndrome) or whole exome sequencing is recommended as it provides the genetic diagnosis in another ~5% cases [6, 15, 51]. This general strategy, however, should be tailored according to the clinical assessment, resource availability, and population-specific considerations. Prenatal genetic diagnosis may be considered in families with a known risk for CNS and rapid turnaround time for the genetic results is possible if the genetic variant is already known.

Knowledge of the etiology helps in management and prognostication. Immunosuppressive therapy should be avoided in patients with a genetic diagnosis; progression to ESKD is also rapid in genetic compared to non-genetic cases. Surveillance and follow-up for extrarenal manifestations can be more targeted with a genetic diagnosis (e.g., endocrine or oncological features in *WT1* or *SGPL1* cases). Genetic testing also enables genetic counseling for the family.

Management

The goals of therapy are to provide good nutrition for optimal weight gain, control edema, and prevent complications like thrombosis, infections, and hemodynamic instability. Since 1970, these goals were achieved in the first months by daily albumin infusions and provision of sufficient nutrition. The ultimate aim is to allow the child to reach a body weight at which preemptive bilateral nephrectomy and dialysis may be performed, followed by transplantation at 1–3 years of age. This “aggressive” approach has been used by many units and resulted in improved survival of children with CNS [52, 53].

However, at least 55% of patients with CNS do not reach ESKD by the age of 2 years, and hence a more conservative and individualized treatment strategy has been recommended by the European Society of Pediatric Nephrology [54]. This protocol, tailored according to the clinical severity of nephrotic syndrome, its complications, as well as response to therapy with ACEi and prostaglandin synthesis inhibitor, has emphasized the possibility of ambulatory care management.

A summary of current management strategies is provided in Table 2.

Albumin Substitution

Since CNS is a heterogeneous group of disorders, urinary protein loss is variable (2–150 g/l), with varying clinical status of the patients. Infants with lower urinary protein losses may have little or no edema, and no albumin replacement may be needed. On the other hand, CNS infants with heavy and prolonged proteinuria are usually critically ill with anasarca, intravascular hypovolemia (slow capillary refill, low blood pressure and /or tachycardia), and often septicemia. Persistent heavy proteinuria can also lead to complications like reduced growth, thrombosis, and infections. While albumin substitutions only temporarily correct hypoproteinemia in patients with heavy urinary losses, it allows control of severe edema and its related complications. Hence, in such cases, protein substitution by albumin or plasma is mandatory.

The decisions on administration of albumin infusions should be based on clinical status instead of target serum albumin levels. After acute resuscitation, albumin infusions should be given together with furosemide. Initially, 20% albumin substitutions may be divided into three infusions a day, starting dose 0.2–1 g/kg (1–5 ml/kg) per infusion, each over 2 h. One to two boluses of

Table 2 Summary of management strategies in children with CNS

Nutrition and fluids
Avoid unnecessary intravenous fluids and salt supplements in stable patients
Adequate nutrition: Calories 120–150 Cal/kg/day; protein 3–4 g/kg/day
Nasogastric tube or gastrostomy often required for proper feeding
Adjust nutrition with declining kidney function
Multivitamins; calcium, magnesium, and potassium supplements may be required
Albumin infusions
Albumin infusions are indicated in unstable patients (severe edema with complications)
Initial 20% albumin dose 0.2–1 g/kg, up to three infusions a day, each over 2 h
Maintenance 20% albumin dose is 1–4 g/kg/day (over 6 h; at night)
During albumin infusion, 1–2 doses of furosemide 0.5–2 mg/kg are given
Medications
Angiotensin converting enzyme inhibitors, angiotensin II type 1 receptor antagonists
After neonatal period: captopril 0.01–0.5 mg/kg twice daily; increase dose gradually
Indomethacin is usually not effective
Anticoagulation prophylaxis, especially with deep vein catheters
Warfarin or enoxaparin
Antithrombin III infusion (50 IU/kg) prior to invasive procedures
Thyroxine substitution, dosed according to thyroxine stimulating hormone levels
Parenteral antibiotics if systemic bacterial infection is suspected
Intravenous immunoglobulin considered in recurrent severe infections
Nephrectomy
Unilateral nephrectomy in selected cases to reduce proteinuria
Routine preemptive nephrectomy is not necessary in stable patients
Consider preemptive bilateral nephrectomy in refractory severe nephrotic state with complications that are difficult to manage (repeated infections, undernutrition and development delay, thrombosis, hemodynamic instability)
Before transplant: Consider bilateral nephrectomy if persistent and significant nephrotic state
Renal replacement therapy
Indication for dialysis: ESKD or after bilateral nephrectomy
Peritoneal dialysis preferred; hemodialysis if peritoneal dialysis contraindicated, or caregiver preference
Transplantation
May be preemptive, provided nephrotic state has resolved
Extraperitoneal grafting is preferred
In autosomal recessive conditions, parents (heterozygous carriers) are suitable donors

intravenous furosemide (0.5–2 mg/kg) are given with each albumin infusion. After few weeks, 20% albumin is given as one infusion (~3–4 g/kg, 15–20 ml/kg) over 6–8 h during the night, allowing for home care during the daytime [9]. Additional furosemide boluses are given during the daytime when the maximum albumin dosing of 4 g/kg/day has been reached. In very edematous patients, IV furosemide is preferred (2–10 mg/kg/day), but in more stable patients, the medication is given orally (2–4 mg/kg/day) [54]. In patients not receiving albumin infusions, diuretics may be given to improve fluid control while exercising caution to avoid intravascular volume depletion.

Management with albumin substitutions should be tailored to the individual patient, and treatment strategies may change within the same patient over time. In some patients, urinary protein losses eventually decline, even in the absence of kidney impairment, resulting in clinical improvement and enabling discontinuation of albumin infusions. This occurred in three of seven CNS children by the age of 10 weeks to 9 months in a single-center study [55]. In a French survey, out of 53 patients, albumin infusions were withdrawn in 10 patients (18%) who had normal eGFR at a median age of 11 (range 5–29) months [56]. Clinicians therefore should exercise clinical judgment when ordering albumin infusions, balancing between the need to avoid intravascular hypovolemia and severe edema with its related complications, versus the need to avoid excessive or unnecessary albumin infusions especially in patients who show clinical improvement and decline in urinary protein losses with time. Albumin infusions can lead to increased costs, prolonged hospitalizations, and need for a central venous catheter with attendant risks of thrombosis and jeopardizing future hemodialysis access.

Home albumin programs have been implemented in some centers [55]. In a report from 17 tertiary units in Europe, out of 68 CNS children, 14 (20%) from 5 centers received albumin infusions at home [57].

Antiproteinuric Medications

In primary CNS, renin–angiotensin–aldosterone system blockade may reduce proteinuria by decreasing glomerular perfusion pressure. Additionally, it possibly also exerts a protective effect on the podocytes and slow renal failure progression. The benefit of ACEi in primary CNS is based on small single-center or retrospective studies. In a recent multicenter survey, 70% (23/33) of CNS children treated with an ACEi had a significant increase in their serum albumin by 3–8 g/l, and a significant reduction of weekly albumin infusion dose by 0–4 g/kg/week, although the latter was not significant among patients without *NPHS1* mutations [57, 58]. The type of *NPHS1* genetic variant (truncating/ frameshift/ splice site versus missense) did not correlate with the response to ACEi. The first anti-proteinuric drug of choice is captopril, because of shorter half-life and hence safer dose titration. It can be commenced after the neonatal period, and the dose should be increased gradually (maximal daily dose in infants <3 months of age is 2 mg/kg, and in older infants 6 mg/kg) [54]. Frequent monitoring of proteinuria, urine output, and kidney function is important. Angiotensin II type 1 receptor antagonists may be used in place of ACEi, but not in combination with ACEi due to increased risk of adverse effects.

Sole treatment with captopril and indomethacin has been reported to cause prolonged and sustained clinical remission in infants with primary CNS [59]. However, opinions differ on whether indomethacin (1–5 mg/kg/day) should be added to ACEi in refractory cases. There were no differences in serum albumin levels or albumin infusion requirements between children on ACEi only versus those on ACEi and indomethacin [57].

Nutrition

Traditionally, infants with severe CNS are given high caloric (120–150 kcal/kg per day) and protein (3–4 g/kg per day) intake [52]. The addition of a whey- and casein-based protein product, glucose polymers, and/or fat emulsions to breast milk or cow milk formulae is required. At weaning, a low-salt diet is ensured. Most patients require a nasogastric tube or gastrostomy to ensure adequate nutritional intake. A complete multivitamin preparation can be given according to the recommended dietary allowances for healthy children of the same age. The daily fluid intake should not be restricted, in order to ensure adequate nutritional intake in infants and to avoid intravascular volume depletion. Nevertheless, if the kidney function deteriorates, modification to this regime should be made.

Additional Medications

In addition to albumin, many other proteins are lost into urine. These include thyroid binding globulin, plasminogen, antithrombin III (ATIII), immunoglobulin G, transferrin, vitamin D binding protein, apoproteins, lipoprotein lipase, and ceruloplasmin [52]. Hypothyroidism, thrombotic events, bacterial infections, anemia, and bone mineral disorders are common. Patients should receive vitamin D3 (10–40 μg/day) and alphacalcidiol (0.1–0.3 μg/day) when parathyroid hormone level is increased. Supplementary magnesium (50 mg/day) and calcium (500–1000 mg/day) are given to ensure normal serum levels. Patients with CNS often have low serum thyroid-binding globulin and thyroxine levels. It is necessary to treat these patients with thyroxine, adjusted according to thyroxin stimulating hormone levels, which remain normal initially and increase during the first months [52].

Urinary excretion of plasminogen and ATIII leads to compensatory protein synthesis, resulting in increased levels of macroglobulins, fibrinogen, thromboplastin, and factors II, V, VII, X, and XII, contributing to hypercoagulopathy. Thromboses and severe coagulation problems have been reported in children with CNS. Administration of warfarin to patients with central catheters has decreased the incidence of severe thrombotic events. Before surgical or vascular procedures, warfarin is stopped and ATIII (50 IU/kg) is given 1 h before the procedure to temporarily correct the ATIII deficiency. Besides warfarin, enoxaparin has been prophylactically used in CNS infants [60]. Urinary loss of transferrin, transcobalamin, and erythropoietin can result in anemia [61]. This can be managed with erythropoietin up to 500 IU/kg/dose, although it may be ineffective in some patients resulting in the need for repeated blood transfusions. On the other hand, some patients with CNS have thrombophilia necessitating the use of low-dose aspirin.

Bacterial infections may be a major problem in CNS. Urinary loss of immunoglobulins and complement factors B and D can lead to increased susceptibility to infections by encapsulated bacteria such as pneumococci. Catheter-related sepsis is common. Infection rates may be 2.4–3 episodes per patient per year, and these are not decreased by prophylactic antibiotics or intravenous immunoglobulins (IVIg), nor increased by the use of central venous catheters [56, 62]. While prophylactic antibiotics are not recommended, prophylactic IVIg may be given to patients with recurrent severe infections and/or low plasma levels of immunoglobulin G. A high index of suspicion remains crucial, especially since symptoms are vague and nonspecific, and plasma levels of C-reactive protein can remain low due to urinary losses. Antibiotic therapy should be started promptly at initial suspicion, and should be broad-spectrum to target nosocomial infections. Response to treatment is satisfactory. Vaccinations should be given as per schedule, including against encapsulated bacteria and varicella. Annual influenza vaccines should be given.

Primary CNS, being genetic in most cases, usually does not respond to immunosuppressive agents like prednisolone or calcineurin inhibitors. Accordingly, only one of 45 patients with CNS and no known genetic defect achieved prolonged remission with steroid therapy [3]. There are few case reports of patients with pathogenic variants in *WT1* and *PLCE1* who show improvement in proteinuria after immunosuppressive therapy [26, 63]. While the mechanism is not clear, there is data to suggest direct effects of immunosuppressive medications on the podocytes [64]. In CNS infants with no identified genetic defects, a trial with steroids or calcineurin inhibitors has been suggested [54]. In patients with CoQ10-related genetic defects, CoQ10 supplementation may decrease proteinuria and slow CKD progression [31, 32]. In secondary CNS due to infections, specific therapy against the infectious agent is often effective.

Nephrectomy and Dialysis

Unilateral nephrectomy, together with ACEi and indomethacin, may be performed to reduce urinary protein losses in infants with CNS [65], so as to decrease the requirement of albumin infusions. According to a recent report, unilateral nephrectomy decreased albumin dose by 5 g/kg/week and increased plasma albumin levels by 4 g/l, but increased serum creatinine from 21 μmol/l to 45 μmol/l [57].

In patients who develop complications associated with the nephrotic state (infections, thromboses, unstable hemodynamics) despite conservative therapy, an “aggressive” approach may be considered. This involved preemptive bilateral nephrectomy followed by peritoneal dialysis or hemodialysis when an infant is 7–10 kg. During dialysis, the general condition improves, with catch-up growth and increase in muscle mass [66]. This allows improvement of the hypoproteinemic, prothrombotic, and malnourished state before kidney transplantation is performed a few months later.

In stable infants with no complications, a conservative approach should be considered. This involves no preemptive nephrectomy before the development of ESKD, so as to delay initiation of renal replacement therapy [67]. In a recent multicenter study, the timing of renal replacement therapy did not influence the survival and growth in children with CNS due to *NPHS1* genetic defects [68]. Peritoneal dialysis is the modality of choice in patients with CNS, but one-third can switch to hemodialysis [58]. Infants with CNS have comparable dialysis outcomes to infants with other renal diseases. The 5-years patient and graft survivals are excellent and comparable to patients with congenital structural anomalies of the kidney and urinary tracts [58].

In Denys-Drash syndrome, progression to ESKD is rapid. Because of the significant risk of Wilms tumor, prophylactic bilateral nephrectomy is performed after onset of dialysis. If nephrectomy has not been performed, tumor surveillance with abdominal ultrasound should be performed every 3 months.

Kidney Transplantation

Renal transplantation is the curative therapy for most patients with CNS, and is often performed at 1–3 years of age using adult-size kidneys. Size discrepancy and the risk of thrombotic complications make this a surgically challenging procedure. Prior to transplantation, it is imperative that the patient does not have the nephrotic state with normal serum albumin levels (hence no longer prothrombotic), in order to minimize risks of thrombosis in the graft.

Recurrence of nephrotic syndrome in the graft is exceptional but has been reported in patients with CNF, who develop anti-nephrin antibodies after transplantation. Treatment of recurrence with cyclophosphamide and plasmapheresis and, more recently, with anti-CD20 antibodies normally leads to remission [69]. Overall, the outcomes of kidney transplantation in CNS are good and comparable to other underlying renal conditions [56, 57, 70]. Additionally, most patients achieved good quality of life and have normal schooling [71]. Patients, who were transplanted as infants, have normal growth and pubertal development, but males may have reduced fertility in adulthood [72]. Chronic allograft nephropathy is a major problem and second transplantations are often needed by young adulthood [68, 70].

Cross-References

- ▶ Chronic Hemodialysis in Children
- ▶ Clinical Aspects of Genetic Forms of Nephrotic Syndrome
- ▶ Genetic Basis of Nephrotic Syndrome
- ▶ Infant Dialysis
- ▶ Inherited Diseases of the Glomerular Basement Membrane
- ▶ Peritoneal Dialysis

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Clinical Aspects of Genetic Forms of Nephrotic Syndrome

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Abstract

Steroid-resistant nephrotic syndrome (SRNS) is a paradigmatic disease to illustrate the major clinical consequences that can arise from genetic testing. Patients with pathogenic variants should be spared inefficient and potentially toxic immunosuppressive therapy; although a partial reduction of proteinuria has been reported with calcineurin inhibitors and/or renin-angiotensin-aldosterone inhibitors in a small number of cases. A better understanding of disease mechanisms may help identifying targeted therapeutic agents, and guide specific management of extra-renal features. Moreover, these patients have a very low risk of recurrence after kidney transplantation. Finally, such diagnosis is crucial for genetic counseling with respect to family planning but also to assess the suitability of relatives as organ donors. Next-generation sequencing approaches are increasingly used as the first step of genetic analyses. Causative pathogenic variants are identified in approximately 30% of children with SRNS and at least 66% in congenital and infantile cases. Among these genes, *NPHS1* and *NPHS2* are by far the two main autosomal recessive genes implicated in SRNS, while *INF2* is the leading cause of autosomal dominant SRNS. Most novel gene variants are rare and involve few families. Moreover, NGS has revealed causative variants in unexpected genes such as ciliary or tubular genes and has widened the phenotypes associated with SRNS. More complex inheritance involving susceptibility variants, oligogenism or interallelic influence of variants have also been described as a cause of SRNS.

Keywords

Proteinuria · nephrotic syndrome · Genetics · FSGS · SMS · NPHS1 · NPHS2 · Podocin · Nephrin

Introduction

Over the past decades, genetic research aimed at unraveling the causes of Mendelian forms of steroid-resistant nephrotic syndrome (SRNS) has led to the description of new pathologies, all of which have in common a primary dysfunction of the podocyte, and are grouped under the term of "hereditary podocytopathies." The classification of these pathologies has become more complicated as variants in new genes have been identified. Indeed, since the discovery of *NPHS1* coding for nephrin in 1998 by Kestila et al. [1], and *NPHS2* coding for podocin in 2000 by Antignac et al. [2], more than 60 genes whose mutations are responsible for SRNS have been discovered (Table 1). The first genes identified appeared to be specific for certain clinical entities such as *NPHS1* whose biallelic pathogenic variants are the main cause of congenital nephrotic syndrome [1], *NPHS2* whose biallelic pathogenic variants have been identified in childhood-onset autosomal recessive (AR)-SRNS [2], or *ACTN4* whose monoallelic pathogenic variants lead to late-onset SRNS or proteinuria with focal segmental glomerulosclerosis (FSGS) [3]. However, subsequent studies have shown, for instance, that *NPHS2* biallelic pathogenic variants could lead to congenital nephrotic syndrome [4], and conversely that less severe *NPHS1* variants were responsible for SRNS beginning in childhood [5]. Thus, hereditary podocytopathies are a

Table 1 Genes implicated in hereditary SRNS and FSGS and associated phenotypes

Gene	Transmission	Encoded protein	Typical histology	Extra-renal involvement; comments
Autosomal recessive isolated SRNS				
NPHS1 [1]	AR	Nephrin	MCD, FSGS	–
NPHS2 [2]	AR	Podocin	MCD, FSGS	–
PLCE1 [36]	AR	Phospholipase C, epsilon 1	DMS, FSGS	–
CD2AP [45]	AR	CD-2 associated protein	FSGS	–
PTPRO [40]	AR	Receptor-type tyrosine-protein phosphatase O	MCD, FSGS	–
MYO1E [39]	AR	Myosin 1E	FSGS	–
COQ8B [165]	AR	aarF domain containing kinase 4	FSGS	
CRB2	AR	Crumbs homolog 2 protein	FSGS	Cerebral ventriculomegaly with congenital nephrotic syndrome
MAGI2 [183]	AR	Membrane-associated guanylate kinase, WW, and PDZ domain-containing 2	FSGS	Congenital/infantile nephrotic syndrome
XPO5 [184]	AR	Exportin 5	MCD	Some response to cyclosporin A, delayed speech development
Autosomal dominant isolated SRNS				
INF2 [46]	AD	Inverted formin 2	FSGS	–
TRPC6 [53]	AD	Transient receptor potential 6	FSGS	–
ACTN4 [3]	AD	α-actinin-4	FSGS	–
WT1 [185]	AD	Wilms tumor protein	FSGS	
LMX1B [71]	AD	LIM homeodomain transcription factor 1B	FSGS	Pathogenic variant affecting the Arg246 residue
ARHGAP24 [71]	AD	Rho-GTPase activating protein 24	FSGS	–
ANLN [72]	AD	Anillin	FSGS	–
X-linked isolated SRNS				
TBC1D8B [73]	XL	TBC1 family member 8B	MCD, FSGS	–
Phenocopies				
COL4A3/ COL4A4 [123]	AD	Collagen IV alpha-3 and 4 chains	FSGS	Hearing impairment
PAX2 [124]	AD	Paired box protein 2	FSGS	–
CUBN [127]	AR	Cubilin	MCD	
TTC21B [125]	AR	Intraflagellar transport protein IFT139	FSGS	Hypertension, tubular basement membrane thickening and FSGS
Syndromic forms				
WT1 [61]	AD	Wilms tumor protein	DMS, FSGS	Wilms tumor, CAKUT in both genders, genital anomalies in 46,XY patients (disorder of sex differentiation/complete gonadal dysgenesis), gonadoblastoma in 46,XY patients

(continued)

Table 1 (continued)

Gene	Transmission	Encoded protein	Typical histology	Extra-renal involvement; comments
LAMB2 [81]	AR	Laminin β2 subunit	DMS	Pierson syndrome: microcoria, abnormal lens, VI, hypotonia, motor delay
LMX1B [110, 111]	AD	LIM homeodomain transcription factor 1B	FSGS, NPS-nephropathy	Nail Patella syndrome: hypoplastic nails/patellae, iliac horns
SMARCAL1 [105]	AR	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a-like 1	FSGS	Schimke syndrome: spondyloepiphyseal dysplasia, T-cell deficiency, cerebral ischaemia, broad nasal tip, hyperpigmented macules
MTTL1 [186]	maternal	$\text{tRNA}^{\text{Leu(UUR)}}$	FSGS	MELAS: myopathy, encephalopathy, lactic acidosis, and stroke-like episode, diabetes, deafness
COQ2 [187]	AR	Para-hydroxybenzoate-polyprenyltransferase	FSGS	Encephalomyopathy, hypotonia, seizures, lactate acidosis
PDSS2 [188]	AR	Decaprenyl-diphosphate synthase subunit 2	no data	Encephalomyopathy, hypotonia, seizures, lactate acidosis
COQ6 [189]	AR	Coenzyme Q10 monooxygenase 6	FSGS	Sensorineural defect, seizures
SCARB2 [119]	AR	Lysosome membrane protein 2 (LIMP-2)	FSGS	Progressive myoclonic epilepsy, cerebral and cerebellar atrophy, peripheral neuropathy, hearing impairment
NEU1 [27]	AR	Neuraminidase 1	FSGS with vacuolated cells	Sialidosis
WDR73 [92]	AR	WD40-repeat containing protein 73	FSGS, DMS	Secondary microcephaly, intellectual deficiency, cortical and cerebellar atrophy, facial dysmorphy, optic atrophy
LAGE3 [98]	XL	L antigen family member 3	FSGS, DMS	GAMOS
OSGEP [98]	AR	O-sialoglycoprotein endopeptidase	FSGS, DMS	GAMOS
TP53RK [98]	AR	TP53 regulating kinase	FSGS, DMS	GAMOS
TPRKB [98]	AR	TP53RK binding protein	FSGS, DMS	GAMOS
GON7 [99]	AR	GON7 subunit of KEOPS complex	FSGS, DMS	GAMOS
YRDC [99]	AR	YRDC domain containing protein	FSGS, DMS	GAMOS
WDR4 [100]	AR	WD40-repeat containing protein 4	FSGS	GAMOS
NUP93 [184]	AR	Nucleoporin, 93-KD	FSGS	GAMOS
NUP107 [184]	AR	Nucleoporin, 107-KD	FSGS	GAMOS
ARHGDIA [101]	AR	Rho GDP dissociation inhibitor α	DMS	Intellectual disability, sensorineural hearing loss, cortical blindness, seizures

(continued)

Table 1 (continued)

Gene	Transmission	Encoded protein	Typical histology	Extra-renal involvement; comments
KANK4 [102]	AR	KN motif and ankyrin repeat domain-containing protein 4	FSGS	Intellectual disability, dysmorphism, atrial septal defect
INF2 [50]	AD	Inverted formin 2	FSGS	Charcot-Marie-Tooth neuropathy, deafness
ITGA3 [1]	AR	Integrin α3 subunit	FSGS	Interstitial lung disease, epidermolysis bullosa

AD autosomal dominant, *AR* autosomal recessive, *CAKUT* congenital anomalies of kidneys and urinary tract, *DMS* diffuse mesangial sclerosis, *FSGS* focal segmental glomerulosclerosis, *MCD* minimal change disease, *GAMOS* Galloway Mowat syndrome

continuum of disorders characterized by glomerular proteinuria of variable severity.

They share common features with the most prevalent immune-mediated forms of idiopathic nephrotic syndrome. Apart from the rare congenital forms, the initial presentation of immune-mediated and hereditary SRNS is generally similar with albuminuria, hypoalbuminemia and edema, and minimal change disease (MCD) or FSGS on biopsy with podocyte foot process effacement by electron microscopy. Family history, multi-drug resistance, progression to kidney failure, and lack of recurrence after kidney transplantation suggest a genetic origin. Indeed, in hereditary forms of SRNS and unlike immune-mediated ones, the protein loss is caused by structural defects of the podocyte, which are not expected to be sensitive to immunosuppressive drugs and are corrected after transplantation.

Next-generation sequencing (NGS) approaches have wholly modified the diagnosis screening algorithms and are recommended as the first step in genetic testing [6–9]. Pathogenic variants are now identified in approximately 30% of children with SRNS [6, 10]; and at least 66% of the cases presenting before 1 year of age [6, 11]. Among these genes, *NPHS1* and *NPHS2* are by far the two main genes implicated in SRNS [6, 10] in European populations, while *COQ8B* (previously *ADCK4*)-mitochondriopathy is frequent in Asia and *APOL1*-related disease in patients of African descent. Further details regarding geographical distribution are provided in chapter "Genetic Aspects of SRNS." Most novel gene variants are rare and involve few families. Moreover, NGS has revealed causative variants in unexpected genes such as ciliary or tubular genes and has widened the phenotypes associated with podocyte gene mutations.

This chapter presents an overview of the clinical features of the main hereditary SRNS, including syndromic forms and advances in the study of familial forms of steroid-sensitive nephrotic syndrome (SSNS), but excluding mitochondriopathies such as the potentially treatable CoQ10-related disorders like late-onset *COQ8B*-associated SRNS that are described in chapter "Mitochondriopathies." Treatment principles, transplant outcomes, and rare cases of recurrence are also discussed. Molecular genetics are detailed in chapter "Genetic Aspects of SRNS."

Isolated SRNS

Autosomal Recessive Forms

NPHS2

NPHS2 biallelic pathogenic variants (Online Mendelian Inheritance in Man, OMIM database identifier#600995) are the main genetic cause of SRNS beginning after 1 month of age and are responsible for 30–40% of AR-SRNS and 10% of sporadic cases [12–15]. The mean age at onset of NS ranges from 2.6 to 3.4 years of age [4, 13] and kidney histology displays MCD or FSGS [2, 16, 17]. Weber et al. found that frameshift or nonsense biallelic variants cause an earlier onset of SRNS than missense variants [13]. In addition, children with the p.Arg138Gln biallelic variant present with early-onset disease [4, 13]. At least two variants, p.Val180Met and p.Arg238Ser are associated with a milder phenotype, including later age at onset of SRNS and at kidney failure [13]. The p.Arg138* variant has been associated with a high incidence of cardiac abnormalities in children [18], although this finding has not been confirmed in patients carrying other variants [19]. Cardiac ultrasound is recommended in the initial evaluation of these children. Most variants cause retention of the mutated protein in the endoplasmic reticulum [20, 21], such as the most prevalent p.Arg138Gln variant representing 32% of the mutated alleles in patients of European descent [13, 20, 22, 23]. Variants that result in podocin endoplasmic reticulum-retention are often associated with earlier SRNS than variants that allow membrane targeting of podocin [4, 20]. This paves the way to chemical chaperones as promising specific therapies to properly target the mutant podocin to the slit diaphragm with the aim of reducing the severity of the SRNS [23].

In addition, *NPHS2* biallelic pathogenic variants have been found in 15–39% of infants with congenital nephrotic syndrome [11, 17, 24], making it the second most common cause of congenital nephrotic syndrome after *NPHS1* variants. The phenotype of these children is less severe than with *NPHS1* variants with a median age at onset of 29 days vs. 2 days, and slower deterioration of kidney function [17].

Moreover, a 2.77-fold increased risk of developing microalbuminuria has been demonstrated in adults bearing the p.Arg229Gln *NPHS2* polymorphism [25]. This non-neutral variant thought to decrease the affinity of podocin for nephrin [26] is present in 3% of the general population with large inter-ethnic variability (7% in Finnish, 0.01% in East-Asian subjects). It has been shown that the association of the p.Arg229Gln variant on one allele with a *NPHS2* pathogenic variant on the other allele induces SRNS that generally occurs later and evolves more slowly than in patients with two pathogenic *NPHS2* variants [6, 14, 26–31]. This association accounts for most cases beginning in adulthood [27, 31]. However, Tory et al. demonstrated that the p.Arg229Gln variant is only pathogenic when associated to C-term variants (exons 7–8) on the second allele [32] through altered podocin dimerization and retention in the endoplasmic reticulum [33]. Therefore, the disease is not transmitted according to a “classical” mendelian autosomal recessive model and genetic counselling is complex [32]. Clinicians must be aware that a [p.Arg229Gln][N-terminal variant] genotype or biallelic p.Arg229Gln variants do not explain the SRNS that may be immune-mediated and therefore may respond to intensified immunosuppression or recur after transplantation [32, 34].

NPHS1

NPHS1 (OMIM#602716) encoding nephrin has been identified as the major gene involved in congenital nephrotic syndrome of the Finnish type (CNF) [1] extensively described in ▶ Chap. 11, “Congenital Nephrotic Syndrome.” However, later findings have broadened the spectrum of renal disease related to *NPHS1* variants since patients with childhood-onset and even adult-onset SRNS may rarely carry *NPHS1* biallelic pathogenic variants [5, 35]. Indeed, the p. Arg1160* biallelic variant may result in a milder phenotype in about 50% of cases with histologic findings consistent with CNF and either mild proteinuria or complete remission up to 19 years of age [17, 24]. Thereafter, Antignac group identified *NPHS1* variants in 10/160 patients (142 families) presenting with SRNS at a mean age of 3 years (0.5–8), and MCD/FSGS lesions [5]. Affected children were compound heterozygous for at least one “mild” missense variant, which exhibited normal trafficking of nephrin to the plasma membrane and maintained its abilities to form homodimers and to heterodimerize with NEPH1. These findings explain the lesser severity of disease observed in these cases.

PLCE1

Hinkes et al. identified *PLCE1* biallelic variants (OMIM#610725) in children with either DMS and early-onset SRNS (median age at diagnosis 0.8 years and kidney failure before 5 years, or FSGS and a later onset of SRNS (2–8 years) [36]. Subsequently, Gbadegesin, from the same group, showed that *PLCE1* truncating variants accounted for 28.6% of cases of isolated DMS in a cohort of 40 patients from 35 families mostly of Turkish origin [37]. An earlier age of onset is reported in patients with *PLCE1* splice-site variants compared with C-terminal truncating variants or missense variants [6]. Interestingly in the original description, two patients bearing truncating variants achieved complete remission when treated early and remained free of proteinuria after several years of follow-up [36]. Later, four families with transiently affected or even asymptomatic family members – although homozygous for truncating mutations – have been published, suggesting an incomplete penetrance of *PLCE1*-associated SRNS [38], an uncommon phenomenon in AR-diseases. A second-locus modifier, yet to be identified, could explain this phenomenon, although variants in 19 candidate genes including 16 phospholipases were ruled out [38]. The reported incomplete penetrance is consistent with the lack of renal phenotype observed in $Plce1^{-/-}$ mice [36].

Less Common Gene Defects Causing AR-SRNS

MYO1E

Mele et al. identified a *MYO1E* biallelic missense variant (OMIM#614131) in a consanguineous family with SRNS, then a truncating variant in 1/29 families with a similar phenotype [39]. The five patients developed SRNS between the ages of 6 and 18 years, and those who had been biopsied had FSGS. In addition, the electron microscopy revealed a thickening and disorganization of the glomerular basement membrane and mesangial matrix expansion. These lesions were very similar to those of *myo1e* knock-out mice. The patients had SRNS but a partial response to cyclosporin.

PTPRO

PTPRO biallelic pathogenic variants (OMIM#600579) were identified by Ozaltin's group in two Turkish consanguineous families [40]. The five affected siblings had developed nephrotic-range proteinuria and 2 had overt-SRNS between the ages of 5 and 14 years with MCD/FSGS and severe foot process effacement, and one progressed to kidney failure at the age of 18 years. Unexpectedly, one 9-year-old child had initial steroid-sensitivity and steroid-dependence with prolonged remission on oral cyclophosphamide and secondary steroid-resistance. Three additional children also presented partial remission with intensified immunosuppression and renin-angiotensin-aldosterone inhibitors (RAASi).

CRB2

Biallelic *CRB2* variants (OMIM#609720) have been identified in four families with isolated SRNS with FSGS diagnosed between 9 months and 6 years of age [41]. Of note, *CRB2* mutations were also detected in five fetuses and one infant with cerebral ventriculomegaly and echogenic kidneys with histopathological findings of congenital nephrosis [42] (▶ Chap. 11, "Congenital Nephrotic Syndrome").

CD2AP

Although a dominant inheritance of *CD2AP* variants (OMIM#604241) was first suggested by mouse models [43], subsequent data provided conclusive evidence that biallelic loss-of-function variants causes childhood-onset SRNS with FSGS in humans [44, 45].

Autosomal Dominant Forms

INF2

Pollak's group identified monoallelic pathogenic variants in the *INF2* gene (OMIM#610982) encoding the inverted formin 2 in 12% of autosomal dominant (AD)-FSGS families [46]. Subsequent studies confirmed that *INF2* variants are the most common cause of AD-FSGS [47–49]. Age at onset of proteinuria ranges from 5 to 72 years and at kidney failure from 13 to 70 years. As expected for AD-diseases, incomplete penetrance was also reported by most authors and some individuals presented with only mild to moderate proteinuria. Conversely, variants in this gene seem to be responsible for <1% of sporadic cases of FSGS due to de novo variants [47–49]. Boyer et al. later demonstrated that *INF2* variants are the main cause of Charcot-Marie-Tooth disease with FSGS (see below) [50]. Most cases of *INF2*-related SRNS have MCD/FSGS on kidney biopsy, although there are some reports of other changes such as IgA nephropathy (a fortuitous association is suspected) [51], or thrombotic microangiopathy in individuals also bearing the atypical hemolytic uremic syndrome CFH-H3 haplotype, this co-inheritance being the most likely explanation for this unusual presentation [52]. No post-transplant recurrence has been reported in patients with *INF2* variants.

TRPC6

Following the first description of a *TRPC6* monoallelic pathogenic variant by Winn's group in a large kindred from New Zealand [53], *TRPC6* variants were evaluated to be responsible for approximately 2–5% of AD-FSGS (OMIM#603965) [54–56]. Affected cases present with nephrotic-range proteinuria in their third or fourth decade with FSGS lesions and develop progressive kidney failure within 10 years after onset. *TRPC6* variants, mostly de novo, have subsequently been reported in children as young as 1 year old. Most of the patients have FSGS lesions although in a small group of patients, diverse biopsy findings such as MCD, C1q nephropathy, IgA nephropathy, and non-specific immune complex changes have been reported [57–60]. It is unclear whether the immune complex depositions are primary or secondary changes due to underlying podocyte injury because of the mutations. Notably *TRPC6* variants have been described in six children, one of whom was a 2-year-old boy with collapsing FSGS and rapid progression to ESKD due to a de novo variant [54, 55, 57, 58]. FSGS due to *TRPC6* variants is not associated with recurrence of disease following kidney transplantation.

WT1

A *WT1* disorder is characterized by congenital/infantile, childhood, or adolescent-onset of a progressive glomerulopathy that does not respond to steroids. Persistent proteinuria is present in the vast majority of cases (>95%), while criteria for diagnosis of SRNS may not be met initially [61–63]. Almost half of the reported patients present initially as an isolated proteinuria, although detailed examination following molecular diagnosis, detect additional findings including disorders of testicular development in majority of 46,XY individuals (for details see below). Accordingly, karyotype analysis is recommended in all prepubertal phenotypic females having a *WT1* diagnosis, despite lack of detectable abnormalities of the external genitalia and/or müllerian structures [7, 62, 64]. Surveillance for Wilms tumor is also recommended as SRNS can precede the tumor by as much as 4 years, be present at the time of tumor diagnosis, or develop years after completion of chemotherapy for the malignancy [62, 65]. The penetrance for WT is estimated to be ~40% in case of exonic variants, i.e., one tumor per 9 years at risk [61]. Therefore, it is important to screen all patients with ultrasonography of the urinary tract every 3 months until the age of 7 years [62, 64]. On the other hand, a few asymptomatic parents heterozygous for the same germline *WT1* variant in their affected offspring have been reported [61, 62, 66].

ACTN4

ACTN4 monoallelic pathogenic variants were the first cause of non-syndromic AD-FSGS identified by the Pollak group [3, 67]. Affected cases present with proteinuria starting in the teenage years or later, show FSGS, and slowly progress to kidney failure in the fifth decade of life, although earlier cases have been seldom reported [3, 68, 69]. Distinctive cytoplasmic electron-dense aggregates in podocytes were described in one study [70] but not confirmed by others. Overall, *ACTN4* variants seem to account for 3–4% of all non-syndromic AD-FSGS. Disease due to *ACTN4* variant is incompletely penetrant and obligate heterozygotes have been reported in multiple kindreds. Recurrence of disease in transplanted kidney in patients with FSGS due to *ACTN4* mutations have not been reported.

Less Common Gene Defects Causing AD-SRNS

ARHGAP24

A loss-of-function *ARHGAP24* variant was identified in one of 310 sequenced families with AD-FSGS [71]. The mother had died of kidney failure at 29 years of age, and 2 offsprings had reached kidney failure at 12 and 20 years of age, respectively. This mutation involved the conserved GAP-catalytic domain of the encoded actin-regulating protein Rho-GAP24 (OMIM#610586).

ANLN

More recently, Gbadegesin et al. identified variants in the *ANLN* gene (OMIM#616027) encoding the actin-binding protein anillin in two families with AD-FSGS [72]. Patients' phenotype was similar to that of other AD-SRNS with age at proteinuria onset varying from 9 to 69 years, FSGS on biopsies and ESKD occurring between 35 and 75 years and no post-transplant recurrence.

X-Linked Forms

TBC1D8B

Dorval et al. identified two different *TBC1D8B* hemizygous missense variants in two unrelated pedigrees with boys affected with early-onset SRNS and FSGS, whereas the mothers and a sister bearing the same variant had no or milder symptoms [73]. This is the first description of an X-linked SRNS.

Syndromic SRNS

SRNS and Urogenital Abnormalities

While various combinations of renal and other findings associated with a *WT1* pathogenic variant were designated as certain syndromes in the past, those designations are now recognized to be part of a phenotypic continuum and are no longer clinically helpful [62]. These include Denys-Drash syndrome (OMIM#194070), Frasier syndrome (OMIM #136680), Meacham syndrome (OMIM#608978), WAGR syndrome (OMIM#194072), isolated nephrotic syndrome type 4 (OMIM#256370), and hereditary Wilms tumor (OMIM#194070). Below the description of the two most common "classical" syndromes belonging to *WT1*-spectrum are nonetheless provided, for the ease of the reader. One should remember, however, that *WT1*-associated disorder presents with incomplete penetrance and variable expressivity. The most common additional findings include disorders of testicular development (with or without abnormalities of the external genitalia and/or müllerian structures) and Wilms tumor, while less common are congenital anomalies of the kidney and urinary tract (CAKUT) and gonadoblastoma.

Denys–Drash syndrome (DDS)

Denys–Drash syndrome (DDS)(OMIM#194080) was originally described as nephrotic syndrome associated with DMS, manifesting within the first year of life [74], predisposition to Wilms tumor and disorders of sex development [61, 62]. Glomerulopathy presents as infantile SRNS with DMS that progresses to kidney failure before 2–3 years of age [61, 63] and is frequently associated to hypertension [75]. Affected patients have a high risk of developing Wilms tumor, either unilateral or bilateral synchronous and/or metachronous, especially in patients with truncating variants [61, 65]. The median age at presentation of the tumor in DDS is 12.5 months, which is younger than in isolated Wilms tumor. Bilateral nephrectomy is recommended at the time of kidney transplantation because the risk of developing the tumor in residual renal tissue is very high. CAKUT are observed in about 10% of individuals with a *WT1* disorder [61, 63, 65]. The most common kidney abnormalities are duplex kidney, horseshoe kidney, and kidney malrotation. The most commonly reported urinary tract anomalies are vesicoureteric reflux, ureteropelvic junction stenosis, and urogenital sinus [62]. 46,XY children typically present with ambiguous or female external genitalia, gonadal dysgenesis, and a predisposition to the development of gonadoblastoma. Nonetheless, few 46,XY fertile males having a *WT1* pathogenic variant have been reported [62]. Patients presenting 46,XY disorder of testicular development (either a disorder of sex development or complete gonadal dysgenesis) should be consented for gonadoblastoma surveillance including prophylactic gonadectomies. 46, XX girls have normal ovaries, normal external genitalia, and are fertile; only two cases of complete gonadal dysgenesis are reported [62].

Frasier Syndrome

Frasier syndrome (OMIM#136680) is classically referred to in a case of late-onset SRNS in a 46, XY individual with complete gonadal dysgenesis and gonadoblastoma [76]. At molecular level, two distinct single-nucleotide variants in the splice donor site of intron 9 of *WT1* gene that change the ratio of +KTS/-KTS isoforms are typically detected. Patients with Frasier syndrome exhibit milder phenotypes with nephropathy onset at adolescence [77]. It is characterized by progressive SRNS with FSGS [61, 63, 78, 79]. Primary amenorrhea, hypergonadotrophic hypogonadism, and streak gonads are associated with a high risk of gonadoblastoma. Management requires a multidisciplinary team, including a geneticist, endocrinologist, urologist, and psychologist. Prophylactic gonadectomy is recommended. Rarely, the KTS mutation in a 46,XY individual may result in disorder of sex differentiation clinically presenting as ambiguous genitalia, extremely high gonadotropin levels, delayed adrenarche, and para-testicular leiomyoma without gonadal dysgenesis [80].

SRNS and Ocular Abnormalities

Pierson Syndrome

Pierson syndrome (OMIM#609049) is a rare AR-disorder involving the basement membrane caused by biallelic variants in *LAMB2*, encoding laminin β2, and characterized by congenital nephrotic syndrome with ocular and brain malformations [81, 82]. In its severe form, nephrotic syndrome manifests from birth with anuria, and is histopathologically characterized by DMS with irregular lamellation of the glomerular basement membrane [81, 83, 84]. Owing to reduced fetal urine output, pulmonary hypoplasia with hypertension and respiratory distress at birth may occur [84]. Congenital nephrotic syndrome may occur with or without ocular abnormalities [83]. Milder presentations include childhood-onset macroscopic hematuria with albuminuria and varied neurodevelopmental and ocular findings [83, 85–87]. Histopathological findings included FSGS with segmental podocyte foot process effacement and thin basement membrane on electron microscopy [87]. Ocular abnormalities, such as microcoria (narrow, nonreactive pupils), were among the original reports of Pierson syndrome. Other features include glaucoma and abnormalities of corneal diameter, iris, and lens [88], retinal thinning, pigmentary changes, detachments and secondary optic atrophy [89]. More recently, the Waters' group described optic nerve hypoplasia as a phenotypic variant of Pierson syndrome [87]. Severe hypotonia at birth has been reported as a feature of Pierson syndrome [84]. Survival owing to initiation of renal replacement therapy early has led to descriptions of psychomotor retardation, severe muscular hypotonia, and blindness in children who survived until the age of 1.3–4.8 years [90]. Alterations in skeletal muscle tissue from one case were compatible with a chronic denervating process [90]. Brain malformations described in later childhood presentations included agenesis of corpus callosum and anterior pituitary hypoplasia, and was associated with seizures, nystagmus, learning difficulties, and growth hormone deficiency [87].

SRNS and Central Nervous System Abnormalities

Galloway-Mowat Syndrome

Galloway-Mowat syndrome (OMIM 251300) is a rare AR-disease consisting of microcephaly associated with SRNS and in some cases with hiatus hernia [91]. GAMOS is clinically and genetically heterogeneous and should be considered in children even in the absence of renal involvement at presentation [92]. Renal abnormalities range from isolated proteinuria to SRNS, the latter, typically presenting in the first months of life and most often rapidly progressing to kidney failure within a few months. Premature death in childhood is common, most often from complications from renal failure. However, there are rarer cases with preserved kidney function in adulthood [93]. Kidney biopsies have revealed various histopathology including DMS, MCD/FSGS, mesangial proliferation, and increased mesangial matrix [93, 94]. Central nervous system abnormalities can either manifest at birth as a reduced head circumference for gestational age (primary microcephaly), irritability, and roving eye movements. If not present at birth, microcephaly can become evident over time (secondary microcephaly) [92]. Clinically, affected patients may present with developmental delay, psychomotor delay, various degrees of intellectual disability, hypotonia, extrapyramidal movements, visual impairment with optic atrophy, and propensity to seizures [95]. Imaging and histologic findings include abnormal cerebral gyri or cerebellar atrophy, lack of cortical stratification, immature cortical neurons, improper orientation of cortical neurons, and glioneuronal ectopias in the leptomeninges, hypomyelination involving the brain stem, spinal cord, and cerebral hemispheres; and complete absence of the internal granular layer of the cerebellum. The dentate gyrus within the hippocampal formation may be absent and the inferior olivary nuclei hypoplastic [96]. Coarsened facies, full lips and cheek, bushy eyebrows, wide nasal root, sloping forehead, pinched nose, micrognathia, and large ears have been described [97]. Disease-causing variants have been identified in ten genes to date: *OSGEP, TP53RK, TPRKB, LAGE3,* and *GON7* encoding the five subunits of the KEOPS complex [98, 99], and *YRDC involved in the same pathway* [99*],* along with *WDR4* [100], *WDR73* [92], and *NUP107* and *NUP133* [96, 100]. *WDR73*, *WDR4*, *NUP107*, *NUP133,* and *GON7* biallelic variants are responsible for more attenuated neurological and renal phenotypes than those associated with variants in *YRDC* gene or in the four other KEOPS genes [92, 98–100].

Less Common Early-Onset SRNS with Intellectual Disability

ARHGDIA Nephropathy

Biallelic *ARHGDIA* missense loss-of-function variants cause infantile onset SRNS with DMS [101]. Intellectual disability was reported in three children, one with sensorineural hearing loss and another with seizures and cortical blindness.

KANK4 Nephropathy

Intellectual disability, facial dysmorphism, and atrial septal defects are extra-renal features of biallelic variants in kidney ankyrin repeat-containing protein 4 (*KANK4*) associated SRNS [102]. KANK2 interacts with ARHGDIA, another infantile SRNS gene regulating the podocyte cytoskeleton [102]. In addition, Gee et al. also discovered rare variants in the related genes *KANK1* and *KANK2* in families with SSNS.

Mitochondropathies that also often present with intellectual disability are discussed in chapter "Mitochondropathies."

SRNS and Peripheral Neuropathy

Charcot-Marie-Tooth Disease

Charcot–Marie–Tooth disease refers to a heterogeneous group of inherited chronic peripheral motor and sensory neuropathies [103]. Boyes et al. reported that *INF2* variants are a major cause of Charcot–Marie–Tooth disease associated with FSGS (OMIM#614455), accounting for approximately 75% of all cases [50]. Progressive distal muscle atrophy with walking difficulties, limited hand function, muscle wasting, and abolition of deep tendon reflexes in the lower and upper limbs with foot and hand deformities are the typical manifestations of the disease. Twelve index patients with *INF2* monoallelic variant presented with proteinuria at a median age of 18 [10–21] years, and kidney failure developed in 11 at a median age of 21 [12–47] years. All patients exhibited lesions of FSGS, but full-blown nephrotic syndrome was noted in only 5 [50]. Median age at onset of peripheral nerve dysfunction was reported to be 13 years (range 5–28). Conduction velocities of the median nerve were intermediate (23–45 m/s). Mild or moderate sensorineural hearing loss was reported in 4 of the 12 families (33%) with an *INF2* monoallelic variant, which is higher than the approximately 5% prevalence reported among patients with Charcot–Marie–Tooth disease [50]. Sural-nerve biopsy specimens display a combination of axonal and demyelinating changes, characterized by marked decrease in myelinated fibers, as compared to age-matched controls, and numerous multilayered "onion bulbs" [50].

SRNS and Skeletal Dysplasia

Schimke Immuno-Osseous Dysplasia

Schimke immuno-osseous dysplasia (OMIM#242900) [104] is a rare AR-multisystemic disorder characterized by spondyloepiphyseal dysplasia, progressive kidney failure associated with FSGS, variable lymphopenia defective cellular immunity, and arteriosclerosis caused by biallelic variants in *SMARCAL1* [105]. Children typically present in early childhood with SRNS and FSGS [106]. Remarkable extra-renal features include growth retardation with radiological evidence of spondyloepiphyseal dysplasia (Fig. 1). Examination of the skin reveals lentigines on the torso. Hypothyroidism, Moya-Moya disease, and transient ischemic attacks are other features. Facial features have been reported to include a triangular shape, a broad nasal bridge, and a bulbous nasal tip. Early death is common and mainly related to opportunistic infections or cardiovascular events [106]. Specific therapy includes infection prophylaxis, hematopoietic growth factors for neutropenia, orthopedic surgery as required, and combined kidney and bone-marrow transplantation [107, 108].

Nail Patella Syndrome

Nail patella syndrome or hereditary onycho-osteodysplasia (OMIM#161200) [109] is a rare AD-disease that affects multiple organs including the limbs, skeleton, kidneys, and eyes. Dysplasia of the nails and absent or hypoplastic patellae are the cardinal features; other features include dysplasia of the elbows, presence of iliac horns, and nephropathy. Renal manifestations occur in 30–50% of patients and typically include proteinuria with or without hematuria and nephrotic syndrome (5–10% cases), which progresses to kidney failure over varying periods of time. Histological

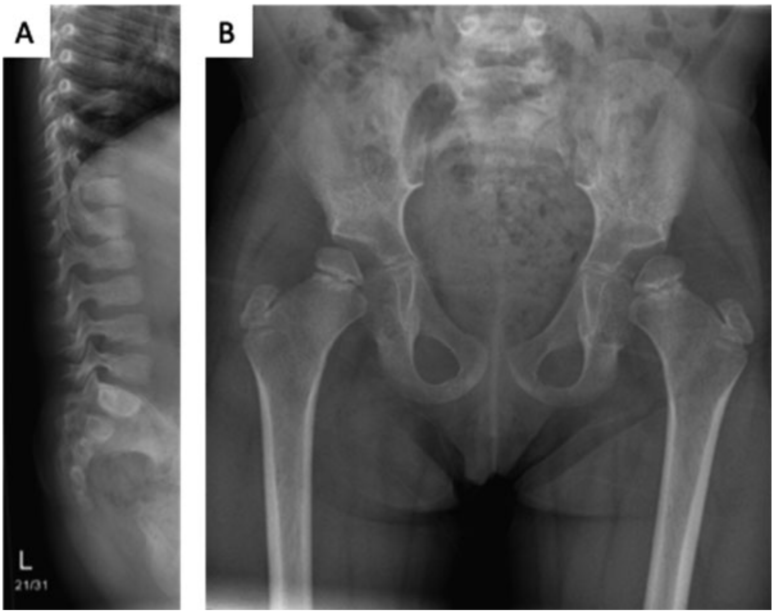

Fig. 1 Schimke immune-osseous dysplasia. (**a**) Platyspondylic vertebrae (**b**) Hypoplastic and laterally displaced femoral capitae. (Courtesy of Dr Aoife Waters)

features include FSGS or renal dysplasia. Electron microscopy shows thickened basement membranes. Additional features include open angle glaucoma and ocular hypertension, which usually develop during adulthood and occur in ~10 and 7% of patients, respectively. Nail patella syndrome is caused by monoallelic loss-of-function mutations in the *LMX1B* gene [110]. Specific *LMX1B* variants cause AD-FSGS but no ultrastructural glomerular basement membrane anomaly suggestive of nail patella-like renal disease, and no extra-renal features [111].

SRNS and Skin Disorders

ILNEB Syndrome

ILNEB syndrome (OMIM#614748) is characterized by interstitial lung disease, congenital or infantile-onset nephrotic syndrome, epidermolysis bullosa, and sparse hair with nail defects [112] (Fig. 2). Kidney biopsies reveal FSGS with trilamellation of the glomerular basement membrane on electron microscopy. Respiratory distress, manifesting from birth with severe reticulonodular changes on chest X-ray has been reported. From early infancy, increasing skin fragility with small blisters and erosions are described. Sparse hair, eyebrows, and eyelashes are observed in association with dystrophic nails and distal onycholysis.

SRNS and Storage Disorders

Disorders of Sphingolipid Disorders

SGPL1 biallelic loss-of-function variants cause sphingosine-1-phosphate lyase insufficiency syndrome (SPLIS, OMIM#617575) characterized by SRNS and primary adrenal insufficiency, immunodeficiency, and neurological defects [113, 114]. Its phenotypic expression is variable, from severe multi-systemic forms, associated with fetal hydrops and early mortality, to isolated single organ disease.

Nephropathy is associated with other genetic disorders of sphingolipid metabolism, such as Fabry and Gaucher disease, and sphingolipid accumulation is reported in glomerular disease of non-genetic origin [115].

Lysosomal Storage Disorder

Sialidosis (OMIM#256550) [27] is a rare AR-lysosomal storage disease due to biallelic loss-of-function variants in *NEU1* encoding for neuraminidase [116]. Disease manifestations can be considered as a milder type I phenotype, characterized by macular "cherry red" spot where myoclonus manifests in adulthood, or the more severe type II that has congenital or infantile onset. Individuals affected by sialidosis type II exhibit typical storage disease features, such as coarse face with low set ears, hepatomegaly, bilateral hearing loss, developmental delay, hip

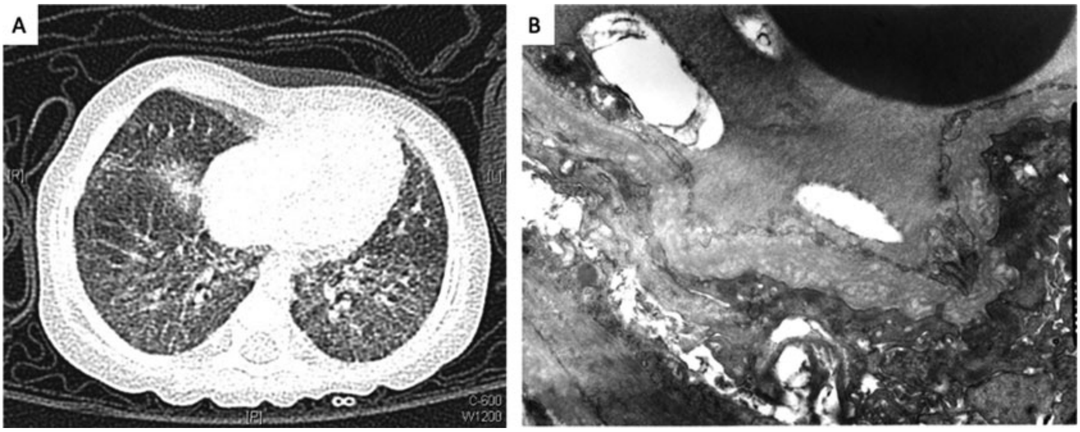

Fig. 2 **Interstitial lung disease, nephrotic syndrome and epidermolysis bullosa (ILNEB).** (**a**) Interstitial pneumonitis (**b**) Trilamellation of the glomerular basement membrane. (Courtesy of Dr Aoife Waters)

dysplasia, and brachycephaly. Bilateral mild frontotemporal atrophy is observed on cranial MRI. Presentation can include antenatal hydrops, infantile onset nephrotic syndrome, childhood isolated proteinuria [117]. Although rare, nephrosialidosis should be considered in children with SRNS if kidney biopsies exhibit severe vacuolar degeneration of renal epithelial cells [118].

Action myoclonus-renal failure syndrome (OMIM#254900) is an AR disease characterized by progressive myoclonic epilepsy associated with renal failure. It is caused by biallelic pathogenic variants in *SCARB2* encoding lysosomal integral membrane protein-2 [119], the receptor for β-glucocerebrosidase, a lysosomal enzyme deficient in Gaucher disease [120]. It typically presents at 15–25 years of age with proteinuria, FSGS and neurological symptoms including tremor, action myoclonus, seizures, and later ataxia, while cognitive function is preserved [121]. Kidney failure usually occurs within 5 years after onset of proteinuria [121].

Reverse Phenotyping and SRNS Phenocopies

With availability of NGS, a number of kidney diseases have now been recognized as phenocopies of SRNS. A phenocopy is a phenotypic trait or disease that resembles the trait expressed by a particular genotype, but in an individual who is not a carrier of that genotype [122]. An example is the report that 10% of patients with FSGS may have molecular diagnosis consistent with Alport syndrome due to *COL4A3, COL4A4, or COL4A5* defects [123]. Similarly, a prevalence of 4% of *PAX2* (papillorenal syndrome gene) monoallelic variants was reported in a cohort of familial non-syndromic FSGS [124]. Another example of phenocopy is the biallelic p.Pro209Leu variant in the ciliary gene *TTC21B*, previously known as a cause of nephronophthisis, and now identified in adolescents with albuminuria, hypertension, and conjunction of tubular basement membrane thickening and FSGS on kidney biopsy [125]. Biallelic *CUBN* variants are a novel cause of podocytopathy with non-nephrotic albuminuria [126]. Variants in *CUBN* encoding cubilin, an intestinal and proximal tubular protein uptake receptor, were previously known to cause the Imerslund-Grasbeck syndrome (OMIM#261100) associating megaloblastic anemia with malabsorption of vitamin B12, and mixed proteinuria. Simons' group identified *CUBN* biallelic pathogenic variants in 39 patients with chronic benign proteinuria up to 1.5 g/day with >50% albumin, and no or minor kidney lesions and normal kidney function [127]. *CUBN*-related proteinuria was often mistaken by clinicians for early-stage SRNS and patients received RAASi that were ineffective. Albuminuria due to cubilin defect is therefore considered a benign condition, and may not require any proteinuria-lowering therapy or kidney biopsy.

Warejko et al. carried out large-scale whole exome sequencing in a cohort of patients with SRNS and reported that up to 5% may have phenocopies of SRNS [128]. Pathogenic variants in *COL4A3-5, AGXT, CLCN5, CTNS, FN1, GLA, and WDR19* genes were identified as phenocopies of podocytopathies in this study. To define better true phenocopies of SRNS, the Romagnani group examined the whole exome of 64 patients with SRNS for pathogenic variants in 298 chronic kidney disease genes, and reassessed the clinical features of the patients (reverse phenotyping). In this cohort, 30% of patients had mutations in podocytopathy genes while 28% had mutations in phenocopy genes identified previously, and also in *FAT1* and *FAT4* [129]. In the 28% patients with mutation in phenocopy genes, clinical reassessment of patients and their families led to identification of subtle features of the genetic diseases suggested by the sequencing data [129]. This study showed clearly that whole exome sequencing combined with reverse phenotyping doubles the diagnostic yield from genetic testing and results in accurate diagnosis and appropriate therapies.

Complex Inheritance

In addition to the Mendelian forms described above, there may also be a complex transmission of SRNS, which may lead to the absence of molecular diagnosis. This is the case, for instance, of susceptibility variants, or rare cases of digenism described as the cause of SRNS. Several genetic susceptibility factors to SRNS/FSGS have been identified in recent years through genome wide association studies (GWAS) or other high-throughput techniques. The most important advance has been the discovery of variants of the *APOL1* gene encoding the apolipoprotein L1 in African-Americans with various nephropathies [130]. The at-risk G1 and G2 alleles are enriched in the biallelic state in West-African and West Indian populations because they confer the resistance to trypanosomiasis [130]. However, the presence of both alleles significantly increases the risk of SRNS in adults and children [130–135]. SRNS associated with two *APOL1* risk-alleles is associated with faster progression to kidney failure, but similar sensitivity to steroids, cyclosporin, or mycophenolate mofetil/dexamethasone compared with other subjects [131, 133]. It is usually not associated with other causative variants in monogenic SRNS genes [134].

Moreover, “additional” heterozygous variants in a gene involved in the development of SRNS have also been found in a few individuals, sometimes associated with a more severe phenotype, suggesting the existence of genetic epistasis [9, 136, 137]. Epistasis describes how gene interactions can affect phenotypes. Examples of digenism and gene modifiers have been reported [9, 136, 138]. Digenism refers to the concept that variants in two distinct genes may interact genetically to manifest a phenotype. For instance, Ars group reported two pedigrees of SRNS/FSGS related to *NPHS2* or *INF2* variants, in which patients carrying an additional *COL4A3* variant had a more severe phenotype [9]. Larger studies are necessary to confirm the role of genetic epistasis in the development of SRNS.

Phenotypic variability is also characterized by the rare occurrence of gene variants in children with nephrotic syndrome that is steroid-sensitive at least at diagnosis and also in children with SRNS (see below) [102, 139]. In such complex cases, it is crucial to ascertain the complete response to steroids or other immunosuppressive drugs [7]. Indeed, RAASi and/or calcineurin inhibitors (CNIs) may enable partial or even nearly-complete remission of proteinuria, even in case of monogenic SRNS. Moreover, the pathogenicity of the identified variants should be confirmed through functional studies, and considering their allelic frequency in different populations.

Familial Forms of Steroid Sensitive Nephrotic Syndrome

SSNS is overwhelmingly due to interaction between different variants in many genes including multiple immune response modulator genes and environmental factors such as lifestyle, diet, exposure to certain pathogens, and other factors [140, 141]. A strong evidence for this is the identification of multiple loci in the HLA region in different populations [142–146]. In addition, anecdotal monogenic causes of SSNS have been reported in the literature despite the fact that 80–90% of all childhood nephrotic syndrome have SSNS response pattern, in contrast, over 60 genes have been identified as single gene causes of SRNS despite the fact that only 10–20% of all NS patients have this pattern of therapy response [140, 141]. It has been estimated that 3% of children with SSNS may have an affected sibling or first degree relative, and there have been published case reports of familial SSNS [139, 147–151]. Similar rates of recurrence in siblings are found for majority of multifactorial (complex) human traits. Dorval et al. reported on the clinical data in families with SSNS comprising of 33 families with possible autosomal recessive mode of inheritance and 29 with autosomal dominant disease [152]. Exome sequencing in this cohort did not identify any monogenic cause of SSNS, however, variants in *HLA-DQA1* were found to be highly linked to the disease in this cohort suggesting that most SSNS are due to complex inheritance and variation in immune response genes are overwhelmingly important in etiology and pathogenesis of SSNS [152]. Few monogenic causes of SSNS have been reported in the literature, including *EMP2*, *KANK1* and 2, *MAGI2* variants [36, 102, 139, 153–156]. Most of these genes shared the features of not causing SSNS exclusively as some individuals in the same family carrying the same variant and other families have variable response patterns ranging from SRNS to frequent relapsing/steroid-dependent course [36, 102, 139, 153–156]. Apart from *FOXP3* that is also associated with T-cell regulation defects [153], products of most of the other genes are not linked to the immune system, but they are important for the structure and function of the podocyte [36, 102, 139, 154–156]. The mechanisms by which defects in podocyte genes will cause steroid-responsive disease is currently under investigation.

Phenotyping

The work-up recommended at diagnosis to search for extrarenal manifestations, suggesting a syndromic form of SRNS is listed in Table 2 [7]. Particular attention should be paid to neurological, eye, ear, genital, and skeletal examinations. Parents and siblings should be offered urinalysis and genetic counseling.

Principles of Treatment

Despite limited experience with treatment of genetic SRNS, clinical practice recommendations of the International Pediatric Nephrology Association for management of children with SRNS including genetic forms have been published in 2020 [7].

Supportive Therapy

Measures to prevent consequences of active disease are detailed in ▶ Chap. 14, "Steroid-Sensitive Nephrotic Syndrome." These include RAASi to reduce proteinuria, cautious use of diuretics, prompt detection and treatment of hypertension, vaccinations, supplements of vitamin D and thyroid hormone if required, and management of chronic kidney disease. Extra-renal features should be managed by multidisciplinary teams.

Table 2 Initial phenotypic work-up of a child with genetic SRNS

Clinical evaluation
Family history • Renal and extrarenal manifestations • Consanguinity
Patient history • Pregnancy and neonatal data • Dipstick at home; urinary protein/creatinine, blood albumin; complete/partial response to therapy • Kidney biopsy results • Genetic results
Physical exam (interdisciplinary evaluation, depending on underlying disease and extrarenal features) • Growth charts: Height/length, weight, head circumference • Dysmorphic features • Ambiguous genitalia • Neurological examination; assessment of cognitive status • Orthopedics: Absent or hypoplastic patella, spondyloepiphyseal dysplasia • Eyes: Microcoria, cataract, glaucoma, optic atrophy, keratoconus, macular spots, lenticonus, nystagmus • Dermatology: Epidermolysis bullosa, icthyosis
Imaging
• Renal ultrasound: Renal echogenicity and size of kidneys • Brain MRI: In case of microcephaly, psychomotor delay, neurological signs • Cardiac ultrasound: In case of *NPHS2*, *CRB2*, *COQ2* biallelic variants • Audiology: Suspicion/risk of sensorineural hearing loss
Specific lab tests
• Lymphocyte immunophenotyping: T cell immunodeficiency (in case of *SMARCAL1* biallelic variants)

Immunomodulators

It has been established in multiple studies that >95% of all genetic SRNS are resistant to glucocorticoids and conventional second line agents (chapters "Treatment of SRNS" and "NS Complications.") Currently, the approach of most pediatric nephrologists is to offer supportive therapy and measures to slow progression of chronic kidney disease. Preclinical studies suggest that glucocorticoids and other immunosuppressive agents may have non-immunomodulatory stabilizing effects on podocyte actin cytoskeleton and the slit diaphragm [157–159]. Xing et al. showed that dexamethasone upregulates the expression of nephrin and tubulin-α in cultured human and murine podocytes [159]. Another study showed that the anti-proteinuric effects of calcineurin inhibitors (CNI) might be mediated by intra-renal hemodynamic changes, inhibition of calcineurin-mediated degradation of synaptopodin, and stabilization of the podocyte actin cytoskeleton [157]. Notwithstanding these effects, most case series emphasize that the overwhelming majority of children with genetic SRNS will not achieve complete remission to corticosteroid or CNI-based therapy, while very few will achieve partial remission [14, 36, 139, 160, 161]. Considering the benefits of partial remission and the impact of delaying progression to kidney failure even for few years on the quality of life of children with SRNS, there may be a place for limited trials of these agents. In a recent review, Gbadegesin et al. proposed a pragmatic approach of discussing the uncertainty of benefits of therapy with parents, coupled with a low threshold for discontinuing therapy if the patient is unresponsive [162]. There is a need for randomized clinical trials to evaluate the usefulness of existing agents, repurpose drugs, and develop novel therapies for the treatment of genetic forms of SRNS.

Targeted Therapy

One of the promises of the advances in genomic science and technology is that identification of genetic causes for SRNS will lead to better understanding of disease mechanisms and identification of targeted therapeutic agents. Despite the fact that we now know >60 monogenic causes of SRNS, only few novel therapeutic targets have been identified. The reasons for this are subjects of recent reviews [162, 163]. Some of the genomic discoveries have led to identification of some potential targeted therapies as outlined below.

Defects in genes that are critical for COQ10 biosynthesis pathway and COQ10 supplementation: Products of multiple genes such as *COQ2*, *COQ6*, *COQ8B* (*ADCK4)*, and *PDSS2* regulate coenzyme Q10 biosynthesis. Variants in these genes have been reported as causes of syndromic and non-syndromic SRNS (see chapter “Mitochondriopathies”) [54, 164, 165]. Supplementation with COQ10, a common food supplement, may therefore represent a specific therapy for patients with CoQ10-related SRNS. Indeed, few cases of partial remission following COQ10 supplementation were reported in these patients [54, 164, 165].

CUBN variants and vitamin B12 supplements: As detailed above, albuminuria due to cubilin defect is considered benign and may not require any proteinuria-lowering agent or kidney biopsy. Whether cubilin inhibition could be a therapeutic strategy in glomerular damaging conditions needs further investigation. The variants associated with isolated proteinuria affect C-terminal residues after the cobalamin-binding domain and do not cause cobalamin deficiency, as opposed to the Imerslund-Grasbeck variants affecting N-terminal residues.

SGPL1 variants and vitamin B6 supplementation: The gene product sphingosine-1-phosphate (S1P) lyase is a vitamin B6-dependent enzyme that degrades sphingosine-1-phosphate in the final step of sphingolipid metabolism [113]. Vitamin B6 supplementation resulted in increased S1P expression and reduced sphingolipids in patients’ derived fibroblasts in some individuals with *SGPL1* variants [113]. However, the effects on disease phenotype is uncertain.

Variants in genes that modulate Rho-like GTPases (RhoA/Rac1/Cdc42) and therapy with mineralocorticoid receptor blockers: These small Rho-like GTPases are known to interact with mineralocorticoids; it is therefore plausible to speculate that mineralocorticoid-receptor blocker such as eplerenone may represent a targeted therapy for patients with SRNS due to mutations in genes that are critical for these pathways [101, 166, 167].

Principles of Transplantation

Children with genetic forms of SRNS often progress to kidney failure owing to a paucity of effective treatment strategies. Kidney transplantation should be offered, with rare exceptions. In patients with severe comorbidities and/or under circumstances with limited medical resources, the decision to proceed with a transplantation can be taken by the medical team after discussing with the families. The long-term post-transplant outcome is excellent [168] and similar to that of children transplanted for other causes [169].

In addition to the risks inherent to any kidney transplant in children, the persistent nephrotic state increases the risk of vascular graft thrombosis. Therefore, uni- or bilateral nephrectomy should be considered at the time of preparing for the kidney transplant [7]. Bilateral nephrectomy is also recommended in case of *WT1* pathogenic variant due to the risk of malignancy. The anticipated recurrence risk after transplantation should be discussed with the family (see below).

Selection of Organ Donors

Living-related kidney donation in the context of genetic SRNS must be preceded by a detailed evaluation of the donor, mode of disease transmission, and by genetic testing and counseling [7, 64]. Heterozygous carriers of autosomal recessive SRNS variants can be kidney donors. Exceptions to that are monoallelic (i.e., heterozygous) defects in collagen IV genes (i.e., *COL4A3*, *COL4A4* and *COL4A5* in females) who should be discouraged from kidney donation because they may develop kidney failure [7, 170]. Also, the parent may rarely bear another pathogenic variant on the second allele, such as the frequent p.Arg229Gln *NPHS2* variant and therefore be at risk of developing SRNS later in life [32, 33]. In cases of autosomal dominant SRNS, living-related kidney donation is also discouraged. Moreover, incomplete penetrance is observed in families with AD-pathogenic variant such as *WT1* variant.

Recurrence of Genetic Forms of SRNS

Recurrence of proteinuria occurs in 30–50% of children with SRNS/FSGS and is the leading cause of pediatric allograft loss, but in only 3–8% in case of identified monogenic disease [13, 15, 171–173]. Indeed, most children with genetic SRNS do not recur after transplantation due to correction of the underlying genetic defect [173]. Post-transplant recurrence of proteinuria has been reported in some patients with variants in *NPHS1*, *NPHS2*, *ACTN4,* and *WT1* genes [174, 175]. *NPHS1* variants are the most-common and unraveled cause of post-transplant proteinuria recurrence [174]. Twenty-five percent of children with *NPHS1* Fin-major/Fin-major variants (that result in the absence of nephrin in the native kidney) experience recurrence of proteinuria at a mean time of 12-months post-transplantation (range 5 days-48 months) [176, 177]. Anti-nephrin auto-antibodies have been identified in recurrent disease in half of cases reported [174, 176]. Therefore, the disease is rather a de novo nephrin antibody-mediated disease than a recurrent SRNS due to *NPHS1* defect. Recurrence of proteinuria has been reported in some patients with *NPHS2* missense variants, as well as in three children with either a missense (p.Trp59Arg) *ACTN4* variant, a splice-site (IVS9+4C>T) *WT1* variant, and *NUP93* biallelic variants [13, 175, 178, 179]. Proteinuria recurrence in patients with *NPHS2* variants have included patients where identified variants were of uncertain significance or single heterozygous variants that do not cause genetic SRNS alone [174, 176]. Bertelli et al. described recurrence in five of 13 (38%) patients with homozygous ($n = 9$) or heterozygous ($n = 4$) podocin mutations compared to 15 of 40 (44%) non-*NPHS2* FSGS patients [180]. However, among the five patients described as having *NPHS2* mutations and recurrence, three had only heterozygous variants predicted to be either benign or polymorphisms with the Polyphen software program [13]. When only patients with biallelic pathogenic *NPHS2* variants are taken into account, the risk of recurrence significantly decreases, concordant with the low recurrence rate of 3–8% reported in other studies [13]. Anti-podocin auto-antibodies have never been identified [180, 181].

In summary, the post-transplant recurrence of genetic SRNS is a very rare phenomenon. While some have a clear pathophysiology, others deserve caution and should lead to a reinterpretation of the cause of post-transplant proteinuria, and of the pathogenicity of the identified variants by an expert geneticist.

Perspectives

Hereditary forms of nephrotic syndrome are far more common than previously thought 20 years ago. The highest rates of variant detection are in patients presenting with SRNS in the first year of life and subsequently decrease among older patients [6]. Most of the cases with hereditary forms of SRNS have a disease onset within early childhood, are resistant to immunosuppressive therapy, and do not recur after kidney transplantation. As a large proportion of patients with SRNS do not have an identified underlying genetic defect, and given the complexity of the pathophysiology of the kidney, numerous further genes are expected to be mutated in SRNS. The most recently identified SRNS genes are rare and only few families are affected. Their discovery does not usually lead to further research due to the small number of families involved. It is therefore difficult to establish the pathogenicity of these variants and the real involvement of these genes in the kidney physiology, in the absence of a robust in vitro or in vivo model. Moreover, in patients with multidrug-resistant SRNS, no post-transplant recurrence, and in whom no mutation has been identified, we can hypothesize one or more deep intronic mutations, not revealed by panel or exome sequencing techniques, as recently demonstrated by comprehensive genome sequencing in a 9-year-old boy with SRNS due to *NUP93* variants: an exonic nonsense and an intronic one leading to aberrant splicing [182]. However, NGS and genome sequencing approaches face the difficulty of determining the causal variant(s) among a great number of potential pathogenic variants. It is also plausible that more complex patterns of inheritance, as described in patients bearing bi- or tri-allelic variants, or as observed with the p.Arg229Gln *NPHS2* variant [32] will be increasingly recognized. Indeed, disease predisposing variants may lead to variable phenotypic expression and penetrance depending upon unidentified environmental and genetic factors [38]. Moreover, common variants in genes expressed in podocytes may account for an increased risk of FSGS and kidney failure observed in selected ethnic groups, as has been described with the *APOL1* gene. To date, several fascinating disorders, such as familial forms of SSNS, connecting podocyte physiology and the immune system, remain incompletely solved.

The constant advances in the understanding of hereditary kidney diseases and renal physiology also open the way for therapeutic innovations. A promising therapy, still explored at a basic level, involves protein chaperones. These drugs redirect the trafficking of missense mutant proteins to the plasma membrane when abnormally retained in subcellular organelles. Additional encouraging results have been obtained with drugs that stabilize the podocyte actin cytoskeleton. It is now clear that genetic diagnosis of cases with SRNS is necessary to allow for accurate genetic counseling, to avoid ineffective therapies and even start early suitable treatment such as ubiquinone in COQ10 deficiency, and hopefully in the near future, to offer specific mutation-based therapies.

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13 Inherited Diseases of the Glomerular Basement Membrane

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Abstract

The glomerular basement membrane (GBM) is a vital component of the filtration barrier of the kidney and is primarily composed of a highly structured matrix of type IV collagen along with laminin isoforms, nidogens, and the heparan sulfate proteoglycans: perlecan, agrin, and type XVIII collagen. Specific isoforms of type IV collagen, the α3(IV), α4(IV), and α5(IV) isoforms, assemble into trimers that are required for normal GBM function. Mutations in these type IV collagen isoforms cause dysfunction of the GBM that varies depending on genotype and sex and is called Alport syndrome. Classic Alport syndrome is characterized by glomerular hematuria with variably progressive chronic kidney disease, sensorineural hearing loss, and ocular findings. Mutations in laminin β2 cause Pierson syndrome manifesting as congenital nephrotic syndrome. Additional disorders with abnormal findings in the GBM on kidney biopsy include nail-patella syndrome and *MYH9*-related disorders.

Keywords

Glomerular basement membrane · Alport syndrome · Pierson syndrome · Type IV collagen · Laminin · Extracellular matrix · Renin-angiotensin-aldosterone pathway

The Glomerular Basement Membrane

Basement membranes are complex extracellular scaffolds that provide support for continuous layers of cells such as the endothelial cells lining blood vessels or epithelial cells lining tubular structures. The glomerular basement membrane (GBM) is one of the most investigated basement membranes in the human body, and this can be attributed to human kidney disease phenotypes such as Alport syndrome. The earliest descriptions of basement membranes came from the work of William Bowman, an English surgeon, anatomist, and histologist who lived between 1816 and 1892 [1]. In an article published by the Royal Society in 1842, Bowman described the structure and use of the Malpighian bodies of the kidney. He described kidney tubules and wrote that “These tubes consist of an external tunic of transparent homogeneous tissue (which I have termed the basement membrane), lined by epithelium” [2]. Together with these careful descriptions, Bowman created detailed sketches based on the appearance of the nephron by early light microscopy. Since those early descriptions, knowledge of basement membrane structure and composition has advanced significantly.

All basement membranes consist of core components that include laminin isoforms, type IV collagen, nidogens, and the heparan sulfate proteoglycans: perlecan, agrin, and type XVIII collagen. From studies of protein interactions in vitro [3] and from studies in model organisms [4], there is evidence of an order to the assembly of basement membranes. This starts with laminin, which is formed from trimers of alpha, beta, and gamma chains that create a cruciform structure (Fig. 1). These trimeric units then polymerize to form networks, which connect to cell surface receptors such as integrins and dystroglycan [3]. The laminin network is a key component for outside-in signaling to cells, and there is a wide range of human pathology associated with genetic defects affecting laminin secretion and assembly. This pathology includes skin, lung, and kidney phenotypes, highlighting the importance of laminin networks in these tissues.

Following assembly of the laminin network, type IV collagen is laid down. There are three distinct collagen IV networks in humans, and these are encoded by the genes *COL4A*1-6. From these six genes, there are six alpha chains which assemble into triple helix trimers with the combinations: α1α1α2(IV), α3α4α5(IV), and α5α5α6 (IV) [5]. The triple helix has a noncollagenous (NC1) domain, a long collagenous domain (consisting of Gly-X-Y repeats), and the 7-S domain. Trimers dimerize at the NC1 domain with a unique sulfilimine bond [6]. This bond requires the enzymatic activity of peroxidasin [7], and this reaction is catalyzed by ionic bromide [8]. Heterotrimers of type IV collagen then polymerize at the 7-S domain to form a network. Type IV collagen is an essential component of all basement membranes and is thought to provide mechanical strength. In the adult GBM, the dominant network is α3α4α5(IV), and by comparison to α1α1α2(IV), this network has a greater number of cysteine residues which may lead to increased resistance to proteolysis by virtue of increased disulfide bonds [9]. The α3α4α5(IV) network is also localized to the cochlea and basement membranes in the eye, and it is the network affected in Alport syndrome.

Laminin and type IV collagen are connected by cross-linking proteins such as nidogen [10]. In addition to these proteins, proteomic studies of extracellular matrix have highlighted many more components [11]. Over 1000 proteins have been annotated in the human matrisome [12], and a repertoire of between 140 and 200 is found in different tissues [13]. It is likely that the tissue specificity for composition relates directly to the function of extracellular matrix in these tissues. In the glomerular matrix, 144 matrisome components have been identified [11, 14], although the functional roles of many of these proteins are unknown. Using gene ontology classification,

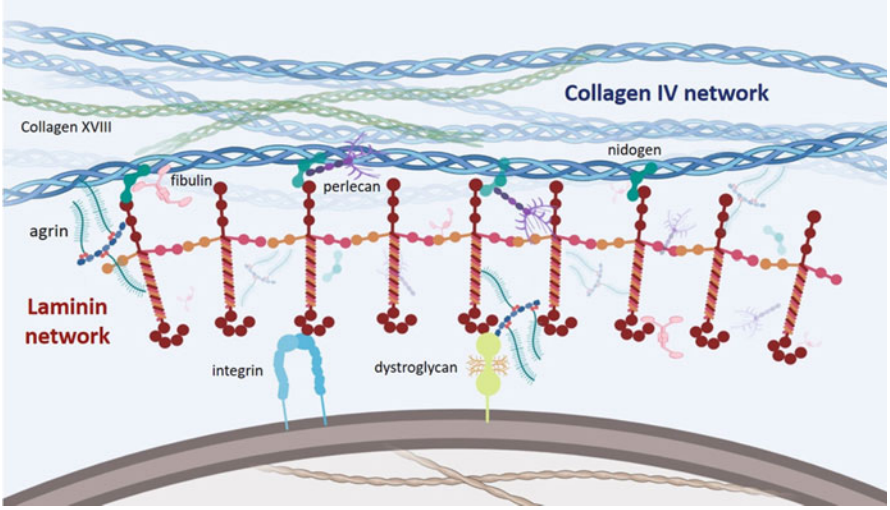

Fig. 1 Major components of basement membranes. Laminin and type IV collagen networks are core components of all basement membranes, and these networks are connected by cross-linking proteins such as nidogen and perlecan

there are approximately 40–50 basement membrane components, and many of these will localize to the GBM [11]. It is harder to know which cells are responsible for producing specific GBM components, although studies of laminin have shown contribution from both endothelial cells and podocytes [15], and for the α3α4α5 (IV) network of collagen IV, this is exclusively podocytes [16].

The GBM is a fusion of two separate endothelial- and podocyte-derived basement membranes that merge during glomerular development. As such, it is thicker than most basement membranes. The average GBM thickness for adult males is 373 nm and for adult females is 326 nm [17]. However, GBM thickness also changes with age from 194 ± 6.5 nm at 1 year to 297 ± 6.0 nm at 11 years [17, 18] in line with overall increases observed in glomerular size with age. Imaging of the GBM with light and electron microscopy is a fundamental requirement of the diagnostic evaluation of kidney biopsies, and kidney pathology is one of the few examples where tissue ultrastructure is a routine component of biopsy evaluation. Indeed, the use of electron microscopy since its origins in the 1950s has made a huge impact on our understanding of the structural features of the glomerular filtration barrier. In studies of the glomerulus using electron microscopy, Karnowsky described a “highly ordered, isoporous substructure of the glomerular slit diaphragm… with alternating, periodic cross bridges extending from the podocyte plasma membranes to a central filament which ran parallel to and equidistant from the cell membranes” [19]. These structural studies were a fundamental basis for building understanding about glomerular function.

Function of the Glomerular Basement Membrane

The glomerular filtration barrier is a specialized capillary wall. It is formed by specialized, fenestrated endothelial cells that line the glomerular capillaries, the podocytes which form a layer of interdigitating cells on the outer surface of the capillaries, and the intervening GBM (Fig. 2). In adults, approximately 180 L of blood per day is filtered by glomeruli, creating an ultrafiltrate which then passes into the tubules of the nephron. The majority of the filtrate is reabsorbed by tubular cells, and the remaining 1–2 L is excreted as urine. Although this process has been appreciated for decades, the mechanism of size-selective filtration is still not fully understood. Furthermore, the contribution of the GBM to filtration is also unclear, reflecting the difficulty in experimentally resolving size selectivity as a function of the glomerulus. The complex and intricate arrangement of podocyte foot processes meant early models of filter size selectivity described in the podocyte slit diaphragm as the major determinant. This concept was supported by measurements that showed the average spacing between podocyte foot processes is comparable to the size of albumin [19, 20]. However, more recent discoveries have questioned this concept, and these more recent studies are described below.

The glomerular albumin sieving coefficient, which is the ratio of the filtrate albumin concentration compared to the plasma albumin concentration, was originally measured as 0.00062 in rats, using fractional micropuncture [21]. This suggests that only a small amount of albumin (22.9 μg/ml filtrate) crosses the filtration barrier and enters the proximal tubule. However, an alternative method of measuring albumin filtration by tracking fluorescently labelled albumin using two-photon microscopy calculated the sieving coefficient as 0.0341, which is 50 times higher [22]. This would demand much greater reabsorption of filtered albumin through the megalin-cubulin endocytic pathway [23] to achieve normal levels of albumin excretion. However, such high levels of albumin reuptake are questioned, and therefore, the true sieving coefficient for albumin in the glomerulus remains controversial.

The endothelial glycocalyx may also contribute to size-selective filtration in the glomerulus. Glycocalyx is a gel-like layer of proteins and glycosaminoglycan (GAG) chains (heparan sulfate, chondroitin sulfate, and hyaluronan), and within the endothelium, it is positioned in the

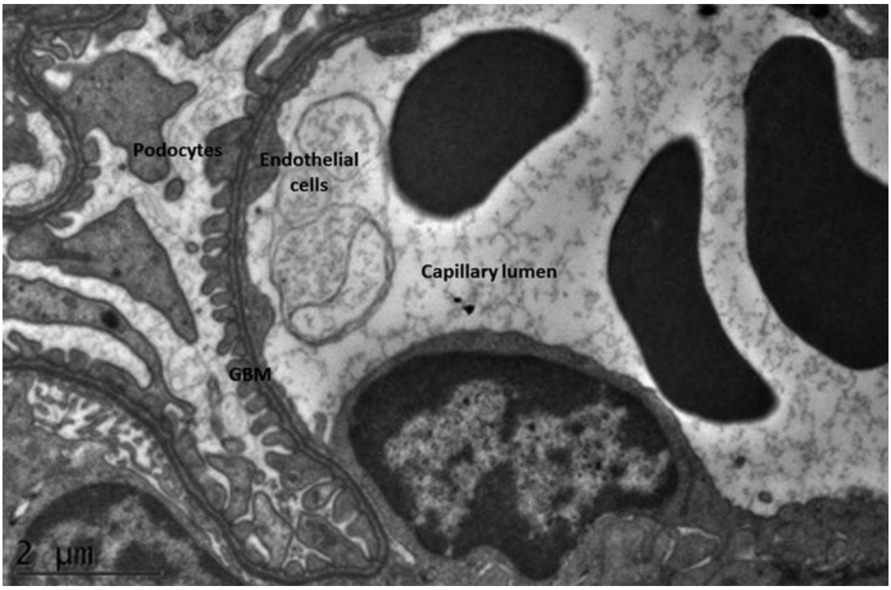

Fig. 2 The glomerular capillary wall is composed of endothelial cells, podocytes, and an intervening glomerular basement membrane (GBM). Red blood cells are visible in the capillary lumen. (Transmission electron microscopy of mouse glomerulus courtesy of Rachel Lennon)

fenestrae and anchors down to the underlying GBM [24]. Enzymatic breakdown of the GAGs that make up the endothelial glycocalyx [25] or displacement of the glycocalyx by hypertonic sodium chloride [26] caused up to a 12-fold increase in albumin filtration. However, the role of the endothelial glycocalyx was questioned by recent in vivo experiments in which the GBM was implicated as the major sieve component of the glomerular filter. Oliver Smithies proposed that the GBM is a gel [27] and should fit with Ogston's 1958 gel permeation principle [28]. If true, the GBM would determine the permeation of macromolecules through the filter in a size-dependent manner through diffusion, whereas water and ions pass through the filter by flow generated from hydraulic pressure [27]. In this model, the fenestrated endothelium and the podocyte slit diaphragms do not act by size selection, but rather provide resistance to the capillary fluid flow. The gel permeation/diffusion model was ultimately supported by a study that used variably sized gold nanoparticles [29]. Large nanoparticles (equivalent in size to IgG dimers) permeated into the subendothelial GBM, but never entered its central region. Smaller nanoparticles (equivalent in size to IgG monomers) were able to partially permeate into the central region. Nanoparticles equivalent in size to albumin (which is much smaller than IgG monomers) were able to permeate across the GBM. When albumin nanoparticles were injected at low concentration, the proximal tubular cells were able to reabsorb these particles, but the injection of high concentrations saturated the reabsorption capacity of the proximal tubules resulting in albuminuria. Intriguingly, the gold nanoparticles used in these experiments frequently aggregated upstream of slit diaphragms at the approximate position of the podocyte glycocalyx. Nanoparticles did not accumulate at the endothelial glycocalyx or at the protein bridges that form the slit diaphragm. These findings led to the conclusion that the GBM (acting as a relatively dense gel) and the podocyte glycocalyx are the two major sites that determine size selectivity in the glomerulus [29]. An explanation for why the podocyte glycocalyx acts as a barrier but the endothelial glycocalyx does not is not clear, and so, further experimental insight is required.

The gel permeation/diffusion model of glomerular filtration appears to stand up to challenge; however, absence of podocyte nephrin precludes slit diaphragm formation and causes massive proteinuria [30, 31], but this does not have a known effect on GBM composition. Rather than suggesting this as evidence for an essential role of the slit diaphragm in size selectivity, this finding may imply a requirement for the slit diaphragm in compression of the GBM to maintain its physiological thickness. Indeed, a gel compression model was recently suggested [32] and proposes that foot process effacement limits GBM compression leading to a subtle, but significant, shift from radial compression to circumferential tension. This leads to a less compressed GBM that has a larger pore size, resulting in passage of larger macromolecules. Genetic models of kidney disease, such as *Lamb2*$^{-/-}$ or *Col4a3*$^{-/-}$ mice, show podocyte foot process effacement and GBM thickening, which is consistent with a requirement for interdigitating podocyte foot processes to maintain compression of the GBM.

The GBM and cellular glycocalyx maintain a net negative charge across the filtration barrier by virtue of heparan sulfate proteoglycans with their glycosaminoglycan (GAG) side chains, which provide the negative charge. This net anionic charge led many to suggest that negatively charged molecules (such as albumin) would be repelled and selectively retained. This theory has been contested by several studies that have depleted HSPGs in the glomerulus but show no defect in glomerular function. In mice depleted of both agrin and perlecan, the net negative charge of the filter was reduced, but proteinuria was not evident [33]. Similarly, removal of heparan sulfate side chains using heparanase infusions did not lead to proteinuria [34]. In zebra fish, mutation of the *ext2* gene, which encodes exostosin glycosyltransferase 2 that polymerizes GAG side chains attached to HSPGs, led to fewer anionic sites in the GBM but did not lead to proteinuria [35]. Taken together, these results suggest that the role of charge selectivity in the glomerular filter is likely to be minor. Despite these convincing findings, anionic molecule tracers have more difficulty in passing the glomerular filter than neutral or cationic tracers [36], and as such, there is likely more to understand about charge selectivity in the glomerulus.

Basement Membrane Pathology

As essential tissue scaffolds, basement membranes are associated with pathology in many organs and tissues. In the skin, blistering disorders such as epidermolysis bullosa are caused by genetic variants in basement membrane genes such as *LAMB3* [37]. In the central nervous system, genetic defects in *COL4A1* and *COL4A2* cause hemorrhagic stroke due to weakened cerebral blood vessels [38]. In the kidney, abnormalities in the GBM accompany a wide spectrum of both genetic and acquired glomerular diseases, and these defects form the basis for disease classifications [39]. However, the mechanisms that lead to abnormal GBM morphology in glomerular disease remain uncertain, and this limits current options for therapy directed at GBM repair [40].

One of the most well-characterized GBM abnormalities is seen with Alport syndrome. This is due to impaired secretion and assembly of the key collagen IV network in the GBM. The initial appearance of the GBM in Alport syndrome is a thin basement membrane [41], and this is also seen in individuals with a single abnormal copy of the Alport genes *COL4A3*, *COL4A4*, or *COL4A5* [41]. The functional consequence of a thin GBM is microscopic and occasionally macroscopic hematuria. How red blood cells traverse glomerular capillary walls remains unclear, although it is likely that this occurs where there are significant splits or defects in the wall [42]. The reduction or absence of the α3α4α5(IV) network of collagen IV in the GBM is thought to limit the resistance of the capillary wall to the mechanical load associated with glomerular filtration. The capillary hydrostatic pressure needs to be opposed by capillary wall resistance, and if the tensile strength of the wall is deficient, this may be sufficient to create splits in the GBM. The consequence of a split GBM is a change in its morphology and composition [43], which further drives the disease progression. The characteristic basket-weave appearance of the GBM is a hallmark feature of Alport syndrome, and this abnormal GBM is associated with podocyte effacement, proteinuria, and a progressive decline in kidney function [43].

Type IV collagen is also the target of the rare autoimmune disorder, Goodpasture syndrome, where autoantibodies are directed toward the α3 (IV) chain. Unlike Alport syndrome, Goodpasture syndrome is a rapidly progressive glomerulonephritis associated with a destructive immune cell infiltration into the glomerulus [44]. Circulating anti-GBM antibodies to the α3(IV) chain serve as a useful diagnostic biomarker, and they deposit within the GBM. Therapy in Goodpasture syndrome is directed at reducing the load and production of autoantibodies with immunosuppression and antibody removal. There is currently no clear genetic basis for Goodpasture syndrome, based on abnormal type IV collagen, although interestingly familial cases have been reported [45].

The laminin network in the GBM is also affected by genetic disorders. The most well described involves variants in *LAMB2* which causes Pierson syndrome [46]. Here, the kidney phenotype is nephrotic syndrome occurring early in life and associated with ocular and neurodevelopmental defects. The GBM is thickened and there is associated podocyte effacement. The laminin 521 network is critical for signaling from the GBM to the podocyte, and these signals transmit via cell surface integrins. Integrin α3β1 is a laminin receptor and a key component of a signaling axis required to maintain intact glomerular function. Indeed, absence of either the β1 or the α3 integrin subunit results in early-onset kidney failure in mice [47]. Integrin β1 has widespread localization and function, and the total knockout is embryonic lethality. However, a podocyte-specific deletion of *Itgb1* in mice, highlights the functional importance of this integrin subunit, with early-onset nephrotic syndrome and thickened, abnormal GBM [48]. To date, no genetic defects in *ITGB1* have been linked to glomerular disease, and this likely reflects its crucial requirement for kidney development. The integrin α3 subunit has more restricted tissue expression, and the total *Itga3* knockout caused a severe lung and kidney phenotype in mice [47] with defects in the GBM. These phenotypes have also been described in patients with *ITGA3* variants associated with kidney, lung, and skin defects [49]. Overall, these examples highlight the importance of the GBM for maintaining glomerular function via a core signaling axis from the laminin network via integrin α3β1 to the podocyte actin cytoskeleton.

Common acquired glomerular disease is also associated with GBM abnormalities. Membranous nephropathy (MN), the commonest cause of nephrotic syndrome in adults, is associated with hugely expanded and distorted GBMs with immune deposits. MN is an autoimmune disorder with autoantibodies targeting podocytes antigens such as PLA2R [50] and THS7DA [51], and in pediatric MN, antibodies to semaphorin-3B have been described [52]. Thickened GBMs are also an early feature of diabetic kidney disease, where abnormal glycosylation and accumulation of advanced glycation end products may contribute. In a range of genetic and acquired glomerular disorders, the progression of disease is associated with increased matrix accumulation in the glomerulus resulting in focal segmental glomerulosclerosis (FSGS) or global glomerulosclerosis. This is a pattern of injury response, which is considered both primary and secondary, but the underlying mechanisms remain uncertain. Proteomic analysis of laser-captured glomeruli identified key molecular changes in different FSGS lesions [53], and these studies may help to further define the mechanisms which lead to FSGS and the ultimate decline in glomerular function across a range of glomerular pathologies.

Alport Syndrome

Familial hematuria was first described as a clinical entity in the early 1900s [54]. In 1927, Alport reported the association of nephritis with deafness in a large family with end-stage kidney disease (ESKD) particularly in males [55]. Numerous descriptions of families with hereditary nephritis were published in the 1950s and 1960s, leading ultimately to the observations that established Alport syndrome as a heritable disorder of type

IV collagen. These seminal events included the identification of unique ultrastructural alterations in Alport GBMs by electron microscopy (EM) [56–58], the observation that Alport GBM exhibited abnormal reactivity with anti-GBM sera directed against antigens associated with type IV collagen [59–61], the mapping of the major Alport locus to the X chromosome [62], the discovery of a type IV collagen gene (*COL4A5*) on the X chromosome [63], and finally the description of *COL4A5* mutations in families with X-linked Alport syndrome [64].

Genetics and Impact on the GBM

Mutations in any of the *COL4A3, COL4A4*, or *COL4A5* genes may adversely affect the formation and composition of affected basement membranes. If any of the α3(IV), α4(IV), or α5 (IV) chains are absent due to severe mutations, then the other type IV collagen isoforms are degraded, and no α3α4α5(IV) heterotrimers are deposited in basement membranes [65]. Milder mutations, generally missense mutations affecting the glycine residues involved in triple helix formation, may lead to the formation of abnormally folded trimers that either are degraded or lead to formation of an abnormal type IV collagen network.

There are three main genetic forms of Alport syndrome: X-linked (XLAS), autosomal recessive (ARAS), and autosomal dominant (ADAS). XLAS is caused by mutations in the *COL4A5* gene and is the predominant form of the disease, accounting for ~80% of affected patients. Affected males are hemizygotes carrying a single pathogenic *COL4A5* variant. Affected females carry a normal *COL4A5* allele as well as a mutant allele and are therefore heterozygotes. Affected females are mosaic for expression of the normal and mutant alleles due to X-inactivation [66]. Approximately 5–15% of patients with Alport syndrome have the recessive form of the disease (ARAS) due to mutations in both alleles of either *COL4A3* or *COL4A4*. These patients are either homozygotes, who have the identical mutation in both alleles of the affected gene (and who may have consanguineous parents) or compound heterozygotes who have inherited different mutations in the affected gene [67]. ADAS caused by heterozygous mutations in *COL4A3* or *COL4A4* was classically thought to be rare, affecting only about 5% of affected patients [68]. With the advent of next-generation sequencing, however, recent studies are suggesting a higher percentage of patients with AS who demonstrate autosomal dominant inheritance than was previously recognized, up to 19–31% [69, 70]. Furthermore, individuals with heterozygous *COL4A3* or *COL4A4* mutations who exhibit isolated, nonprogressive microscopic hematuria previously known as thin basement membrane nephropathy (TBMN) are now included in the category of ADAS [71]. Digenic Alport syndrome is a newly recognized genetic form of Alport syndrome with mutations in two of the *COL4A3*, *COL4A4*, or *COL4A5* genes (10, 29), and only a few patients have been reported.

Over one thousand different mutations in the *COL4A5* gene have been identified in patients and families with XLAS [72]. Mutations can be found along the entire 51 exons of the gene without identified hot spots. About 10–15% of *COL4A5* mutations occur as spontaneous events; therefore, a family history of kidney disease is not required for a diagnosis of XLAS. Reported mutations include large rearrangements (~20%), small deletions and insertions (~20%), missense mutations that alter a glycine residue in the collagenous domain of the α5(IV) chains (~30%), other missense mutations (~8%), nonsense mutations (~5%), and splice site mutations (~15%) [72–75]. The *COL4A5* genotype has a powerful effect on the phenotype of XLAS in affected males [74–76]. The position of a glycine substitution may also affect the XLAS phenotype, with 5′ glycine missense mutations causing a more severe phenotype than 3′ glycine mutations [75]. The number of side-chain carbon atoms in the substituting amino acid also influences the phenotype associated with a glycine substitution [77].

A similar variety of mutation types has been described in patients and families with ARAS [67, 73, 78–80]. There are also genotype-phenotype correlations in patients with ARAS where nonsense mutations or mutations resulting in stop codons are associated with early-onset renal failure [67]. Genotype-phenotype correlation in patients with ADAS is unknown. It is unknown why some patients with heterozygous *COL4A3* and *COL4A4* have progressive CKD whereas others have only thin GBMs and hematuria.

Clinical Features

Sex and genotype are the major determinants of the severity of kidney, cochlear, and ocular diseases in Alport syndrome (Table 1). Males with XLAS and patients of either sex with ARAS inevitably progress to ESKD, and the majority develop sensorineural deafness [67, 74]. The pacing of these events is influenced by the nature of the underlying disease mutation. While the majority of women with heterozygous *COL4A5* mutations have mild disease manifestations, ESKD and deafness develop in a significant minority [81]. Patients with ADAS exhibit relatively slow progression of kidney dysfunction, whereas hearing and ocular findings are much less common than in XLAS and ARAS [69, 82, 83].

Kidney

Persistent microscopic hematuria is the cardinal clinical feature of Alport syndrome, occurring in 100% of males with XLAS, 95% of females with XLAS, and all patients with ARAS [74, 81]. Hematuria is likely present from infancy in XLAS males and in patients with ARAS. Episodic gross hematuria is not unusual, especially during childhood [84]. Some children with Alport syndrome have virtually constant gross hematuria.

Overt proteinuria typically appears during later childhood or adolescence in boys with XLAS and in patients with ARAS. Affected children first demonstrate microalbuminuria, which progresses to overt proteinuria and even nephrotic syndrome with time [84–86]. About 75% of women with XLAS ultimately develop proteinuria of some degree [81]. Most children with Alport syndrome have normal blood pressure, but hypertension is common in adolescent males with XLAS and adolescent patients with ARAS.

In untreated males with XLAS, the probability of ESKD is 50% by age 25, 80% by age 40, and 100% by age 60 [74]. *COL4A5* genotype has a powerful effect on rate of progression to ESKD in XLAS males. Large deletions and nonsense mutations confer a 90% probability of ESKD before age 30, compared to a 70% risk with splice site mutations and a 50% risk with missense mutations [74]. A second cohort study demonstrated a median age of ESKD of 20 years for patients with truncating variants in *COL4A5* and 40 years for those with non-truncating variants (45). Risk factors for earlier ESKD also include absence of α5(IV) in the GBM and hearing loss [76]. The natural history of ESKD in ARAS is similar to males with XLAS [67]. It takes ~50 years for 50% of ADAS patients to develop ESRD, twice as long as males with XLAS [69, 82].

Women who are heterozygous for *COL4A5* mutations demonstrate widely variable disease outcomes with some women demonstrating only lifelong asymptomatic hematuria while others develop ESKD [87]. According to one study of a large cohort of women with XLAS, the risk of ESKD was 12% by age 45, 30% by age 60, and 40% by age 80 [81]. The explanation for the wide variability in outcomes for women with XLAS is unclear but likely multifactorial. Unlike in males with XLAS, genotype does not correlate with phenotype in women with XLAS, perhaps as a result of the overwhelming influence of random X chromosome inactivation on disease course in affected women [66, 81]. Risk factors for ESKD in women with XLAS include proteinuria and sensorineural deafness [81, 88]. Further studies are required to develop accurate methods to predict the risk of progressive kidney disease in women with XLAS.

Hearing

Hearing is normal at birth and during early childhood. Symmetrical deficits in sensitivity for high-frequency sounds often become detectable by audiometry in late childhood. Over time, the hearing deficit progresses into the frequency range of

Table 1 Clinical and biopsy features of Alport syndrome by genetic type

	X-linked		Autosomal recessive	Autosomal dominant
	Males	Females		
Gene	*COL4A5*	*COL4A5*	*COL4A3 or COL4A4*	*COL4A3 or COL4A4*
Clinical features (lifetime risk)				
Hematuria	100%	95%	100%	?
Proteinuria	100%	75%	100%	?
ESKD	100% (50% by age 25 years, 80% by age 40 years)	~30% by age 60 years	100%	<1% if no risk factors ~20% if risk factors are present
Sensorineural deafness	80–90% (50% by age 15 years, 75% by age 25 years)	10% by age 40 years 20% by age 60 years	>65%	Rare
Eye findings	~40%	~15%	~40%	Rare
Kidney biopsy				
GBM width	Early: thin Late: thick and thin	Early: thin Late: thin only or thick and thin	Early: thin Late: thick and thin	Early: thin Late: thin only or thick and thin
α5(IV) in GBM	Absent in ~80%	Mosaic in ~60–70%	Absent in majority	Present
α5(IV) in Bowman's capsule and distal tubular basement membrane	Absent in ~80%	Mosaic in ~60–70%	Present	Present

conversational speech. Because the deficit typically does not exceed 60–70 dB and speech discrimination is preserved, hearing aids are effective in most affected individuals. In males with XLAS, the probability of hearing loss is 50% by age 15, 75% by age 25, and 90% by age 40 [74]. *COL4A5* genotype influences the probability of hearing loss, with 90% of those with deletions, nonsense mutations, and splice site mutations exhibiting deafness before age 30, compared to 60% of those with missense mutations [74] (48). In women with XLAS, the probability of hearing loss is 10% by age 40 and 20% by age 60 [81]. The majority of patients with ARAS develop deafness, although precise data on timing is not available [67, 89].

Eye

Anomalies of the lens, retina, and cornea are common in Alport syndrome, especially among males with XLAS and patients with ARAS, often becoming apparent during adolescence and young adulthood [83]. About 15% of males with XLAS exhibit anterior lenticonus, in which the central portion of the lens protrudes into the anterior chamber [74]. While this lesion is often asymptomatic, it may be associated with reduced visual acuity, and cataracts and even rupture of the lens may occur. Alteration of retinal pigmentation, consisting of whitish-yellowish perimacular flecks, occurs in about 15% of males with XLAS [74, 90], often in association with lenticonus. Corneal abnormalities include recurrent corneal erosions [91, 92] and posterior polymorphous dystrophy [93]. Patients with severe *COL4A5* mutations are more likely to develop perimacular dot-and-fleck retinopathy [94]. Approximately 17% of patients with ARAS have ocular findings, whereas this is rare in patients with ADAS [89].

Others

The association of XLAS with smooth muscle tumors (leiomyomas) of the esophagus; the tracheobronchial tree; and, in women, the external genitalia has been described in several dozen families [95–97]. Symptoms such as dysphagia, postprandial vomiting, epigastric or retrosternal pain, recurrent bronchitis, dyspnea, cough, and stridor often appear in late childhood. The Alport syndrome-diffuse leiomyomatosis complex arises from a contiguous gene deletion on the X chromosome involving exon 1 of *COL4A5*, the common promoter region that regulates gene expression of *COL4A5* and *COL4A6*, and the first two exons of the adjacent *COL4A6* gene [95].

Mental retardation, midface hypoplasia, and elliptocytosis have been described in a small number of males with XLAS who carry deletions that extend downstream of the 3′ end of the *COL4A5* gene [98, 99]. Finally, abnormalities in arterial vessels have been described in males with Alport syndrome including aortic root dilatation and aneurysms of the thoracic and abdominal aorta [100].

Pathology

The clinical features of Alport syndrome originate in changes in basement membrane structure and function initiated by absence or abnormalities of α3α4α5(IV) and α5α5α6(IV) networks.

Kidney

Light microscopic abnormalities are unusual in children with Alport syndrome who are less than 5 years of age (Fig. 3A). Mesangial hypercellularity and matrix expansion, and eventually focal segmental glomerulosclerosis, are common in older children and adolescents, especially boys. Tubular atrophy and interstitial fibrosis develop progressively after age 10 in boys with Alport syndrome [101]. Prominent interstitial foam cells may be present [43] (Fig. 3B). Some patients with Alport syndrome may have FSGS lesions on kidney biopsy [102–104].

Electron microscopy may reveal pathognomonic changes, depending on the patient's age and sex. The earliest abnormality is diffuse thinning of the GBM (Fig. 3C). In some families with Alport syndrome due to *COL4A5* mutations, GBM thinning is the only ultrastructural abnormality. However, the great majority of boys with XLAS and both boys and girls with ARAS develop the classic Alport GBM lesion, consisting of diffuse thickening accompanied by "basket-weave" transformation of the lamina densa, intramembranous vesicles and densities, scalloping of the epithelial surface of the GBM, and foot process effacement (Fig. 3). The percentage of GBM displaying this lesion increases progressively with age in boys with XLAS [105]. Females with XLAS display a range of GBM alteration, from focal GBM attenuation to diffuse thickening and basket weaving, and there is no consistent correlation of GBM findings and age [105].

Routine immunofluorescence is normal or shows nonspecific immunoprotein deposition. Because disease-causing mutations result in abnormal expression of α3α4α5(IV) and α5α5α6 (IV) networks in basement membranes in most Alport patients, specific immunostaining for type IV collagen α chains is useful for both diagnosis and differentiation of XLAS and ARAS [106] (Figs. 4 and 5). Expression of the α3(IV), α4(IV), and α5(IV) chains is completely absent in ~80% of XLAS males, and 60–70% of females with XLAS exhibit mosaic expression of these chains [106] (Fig. 6). In most patient with ARAS, GBM is nonreactive with antibodies to the α3(IV), α4(IV), and α5(IV) chains, and Bowman's capsules and tubular basement membranes are also negative for the α3(IV) and α4(IV) chains [107] (Fig. 5). However, immunostaining for α5(IV) chains in Bowman's capsules and tubular basement membranes is present, because in these basement membranes expression of α5α5α6(IV) networks is preserved. It is important to note that altered immunostaining for the α3(IV)-α6(IV) chains in patients with XLAS and ARAS is not age-dependent. Therefore, this method can provide diagnostic information even in patients who are too young to display characteristic abnormalities in GBM ultrastructure.

Normal epidermal basement membranes (EBM) express the α5α5α6(IV) network but not the α3α4α5(IV) network. EBMs show negative staining for α5(IV) chains in about 80% of

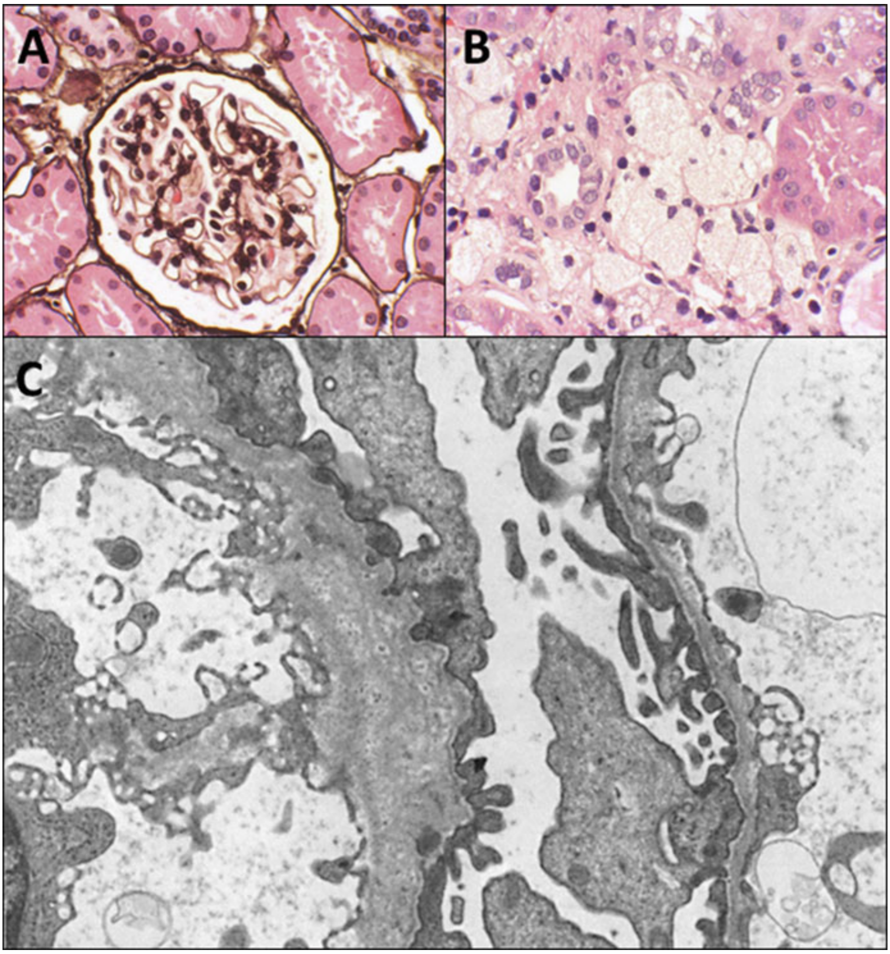

Fig. 3 (**a**) 9-year-old boy with proteinuria, hematuria, family history of Alport syndrome. The glomeruli appeared unremarkable by light microscopy (Jones' silver stain, ×400). (**b**) Multifocal interstitial foam cells, a finding that may be present even in early Alport syndrome due to longstanding low-grade proteinuria, and also seen in patients with marked proteinuria due to other causes. This is the same patient as in A, with minimal interstitial fibrosis (hematoxylin and eosin, ×400). (**c**) Alport syndrome electron micrograph. Right: Thin GBM. Left: Thickened GBM showing splitting and lamellation of the lamina densa with effacement of podocyte foot processes. Lead citrate and uranyl acetate stain (×11,250). (Images **a** and **b** courtesy of Dr. Agnes Fogo. Case kindly shared by the Laboratory for Kidney Pathology, Nashville, TN)

XLAS males, and mosaic expression of α5(IV) is observed in 60–70% of XLAS females, allowing diagnosis of XLAS by skin biopsy [108, 109]. Skin biopsy is not useful for diagnosis of ARAS, since expression of the α5α5α6 (IV) network in EBM is normal [107]. Skin biopsy has fallen out of favor for diagnosis of Alport syndrome given these limitations as well as the increased accessibility of genetic testing.

Diagnostic Considerations

Accurate diagnosis of Alport syndrome and differentiation of this condition from other causes of familial and sporadic glomerular hematuria are based upon careful clinical evaluation, reliable pedigree data and thoughtful consideration of the relative merits of kidney biopsy and/or genetic testing. In a child with isolated hematuria and no

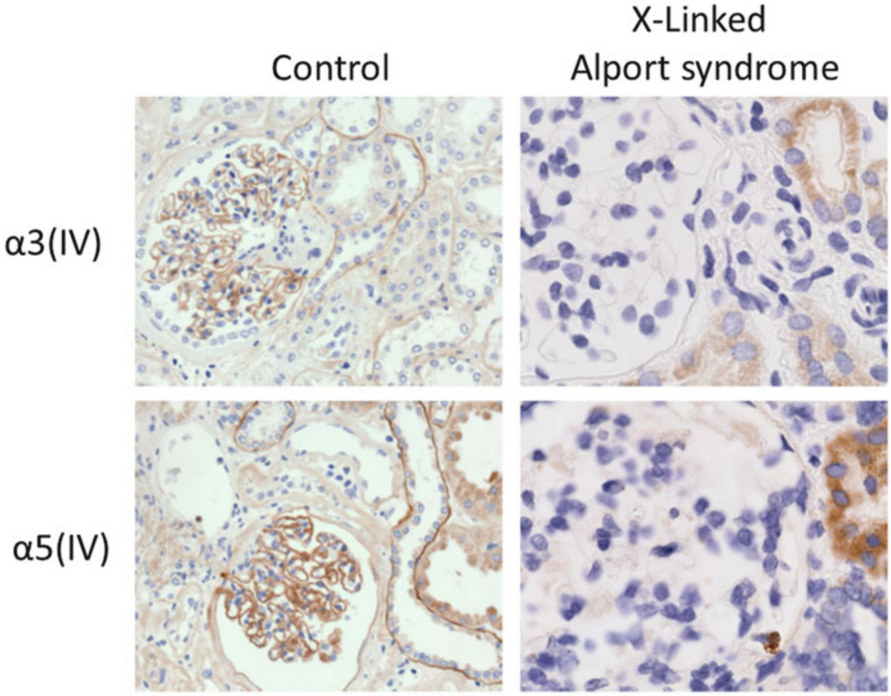

Fig. 4 Immunohistochemistry of normal kidney and male with X-linked Alport syndrome. In the normal kidney, α3(IV) and α5(IV) are present within the GBM. In patient with Alport syndrome, α3(IV) and α5(IV) are absent. (Images courtesy of Dr. Jan Ulrich Becker)

family history of hematuria, the differential diagnosis of glomerular hematuria includes Alport syndrome, IgA nephropathy, membranoproliferative glomerulonephritis, membranous nephropathy, lupus nephritis, postinfectious glomerulonephritis, and IgA vasculitis. Associated clinical findings (e.g., rash, arthritis) or laboratory findings (e.g., hypocomplementemia) will suggest diagnoses other than Alport syndrome in many of these patients. In a child with isolated hematuria and a positive family history of hematuria in the absence of a history of ESKD, two rare causes of familial hematuria associated with macrothrombocytopenia, Epstein and Fechtner syndromes, can be excluded if the platelet count is normal. Familial IgA nephropathy [110] and membranoproliferative glomerulonephritis [111] are uncommon causes of familial hematuria.

While results of hearing evaluation are likely to be normal in young children with Alport syndrome, audiometry may be very useful in children over 6–8 years of age, especially boys. Ophthalmologic assessment may also provide valuable information, although ocular lesions are more prevalent in Alport patients with advanced disease and less likely to be present in the population of young patients in whom differential diagnosis of hematuria may be more difficult.

Tissue studies can complement clinical and pedigree information that is insufficient to clearly differentiate Alport syndrome from other diagnoses. Skin biopsy with immunostaining for the α5(IV) chain, as described above, may be diagnostic, especially when clinical and pedigree data strongly suggest a diagnosis of XLAS. Normal expression of the α5(IV) chain in epidermal basement membrane can be explained in several ways: (1) the patient has XLAS, but his or her *COL4A5* mutation does not abolish α5(IV) expression; (2) the patient has a form of Alport syndrome (ARAS or ADAS) in which expression of α5(IV) in epidermal basement membranes is not affected; or (3) the patient does not have Alport syndrome. While skin biopsy is useful only if it

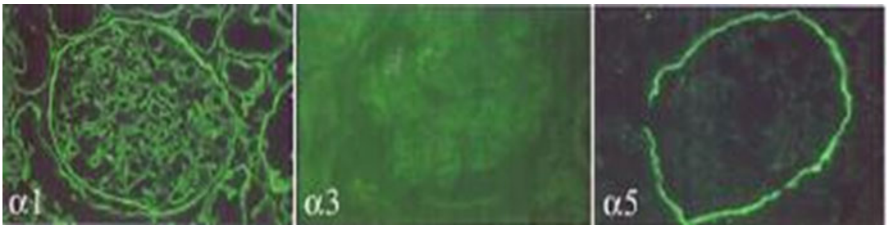

Fig. 5 Immunofluorescence microscopy of glomeruli from patient with autosomal recessive Alport syndrome. The α3α4α5(IV) trimer is absent in the GBM; however, the α5α5α6(IV) trimer is visible in Bowman's capsule

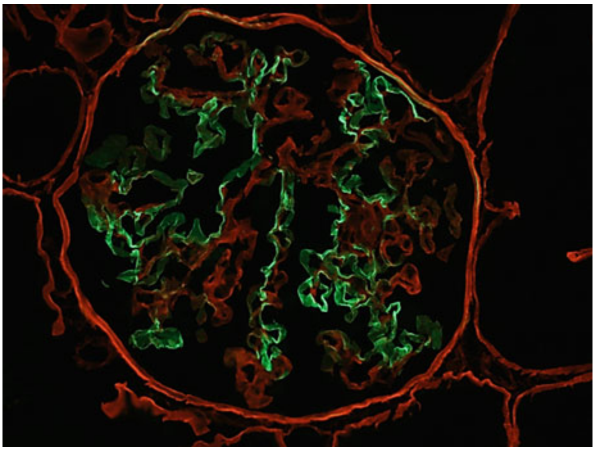

Fig. 6 Immunofluorescence microscopy of glomerulus from women with X-linked Alport syndrome. Red identifies α2(IV) in the GBM. Green identifies α5(IV) in a mosaic pattern in the GBM reflective of X-inactivation. (Image courtesy of Dr. Lihong Bu)

provides definitive confirmation of a diagnosis of XLAS, renal biopsy carries the advantage of enabling the diagnosis of XLAS, ARAS and non-Alport kidney disease, particularly if type IV collagen immunostaining is applied.

Mutation detection rates of 80–90% are attainable in males with XLAS using direct sequencing of *COL4A5* [112]. Comparable data for detection of *COL4A3* and *COL4A4* mutations in patients with ARAS are lacking. Next-generation sequencing (NGS) allows for rapid simultaneous analysis for mutations in *COL4A3*, *COL4A4*, and *COL4A5* [70]. The genetic testing approach may vary based on the level of suspicion of a diagnosis of Alport syndrome. When suspicion is high, NGS of *COL4A3*, *COL4A4*, and *COL4A5* is indicated. When suspicion of a diagnosis of Alport syndrome is moderate or low, a broad NGS panel including focal segmental glomerulosclerosis causative genes or even whole exome sequencing should be considered (82). Commercially available genetic testing for mutations in *COL4A3, COL4A4*, and *COL4A5* is available in many countries. Since the finding of a heterozygous mutation in *COL4A3* or *COL4A4* cannot guarantee a benign prognosis [69, 70, 113, 114], the need for follow-up examination of such patients is vital.

Treatment

The goal of treatment in patients with Alport syndrome is to slow the progression of kidney disease and delay the need for renal replacement therapy. Inhibition of the renin-angiotensin system (RAS) is the mainstay of treatment. These agents reduce glomerular hyperfiltration and inhibit profibrotic pathways within the kidney.

RAS blockade in mouse models of Alport syndrome leads to a doubling of lifespan in animals treated early in their disease course [115]. Uncontrolled studies in patients with Alport syndrome have shown that RAS blockade can transiently reduce proteinuria [116, 117]; however, it remains unclear if this can predict loss of glomerular filtration rate (GFR). Subgroup analysis of 30 children with Alport syndrome in a larger multicenter, randomized, double-blind study comparing losartan with placebo or amlodipine in proteinuric children demonstrated a significant reduction in proteinuria in the losartan-treated group [118]. An extension of this study showed comparable efficacy of either enalapril or losartan in reducing proteinuria in children with Alport syndrome [119]. A retrospective review of 79 children with Alport syndrome showed a significant decrease in proteinuria over the first 2 years of treatment that was stable in years 3–5 [120].

Two retrospective registry studies show the strongest evidence for treatment with RAS blockade. A report from the European Alport Registry, which included 283 patients followed over 20 years, compared kidney outcomes in Alport syndrome patients treated with RAS inhibition starting at various disease time points: microalbuminuria, proteinuria, or chronic kidney disease (CKD) stages III–IV [121]. This study demonstrated a delay in renal replacement therapy by 3 years in the treated CKD group and by 18 years in the treated proteinuric group [121]. A second report from the Japanese Alport Registry reported outcomes in 430 males with XL Alport syndrome [76]. They reported that patients treated with RAS inhibition reached ESKD an average of 22+ years later than those who were untreated. There were benefits to treatment for patients with both truncating and non-truncating mutations. A randomized controlled trial of ramipril versus placebo in children with early Alport syndrome (hematuria alone or albuminuria between 30–300 mg/g) was unfortunately unable to recruit sufficient patients, and an additional open-label treatment arm and retrospective control arm were added to enhance interpretation of results [122]. Over 6 years of follow-up, there was a 50% decreased risk of proteinuria progression in the ramipril-treated group; however, results were not statistically significant. Importantly, no safety issues were identified in over 200 patient years of treatment.

Clinical practice guidelines have been developed to guide treatment of patients with Alport syndrome, including heterozygous females [123]. Treatment with angiotensin converting enzyme inhibitors or angiotensin receptor blockers should be initiated for boys with XL Alport syndrome or boys and girls with AR Alport syndrome at the time of diagnosis after thorough discussion of risks and potential benefits of treatment with parents. Treatment is recommended for children with AD Alport syndrome or girls with XL Alport syndrome when they develop any albuminuria.

A number of novel treatment strategies have been shown to slow progression of kidney disease in mouse models of Alport syndrome including transforming growth factor beta 1 inhibition [124], chemokine receptor 1 suppression [125], administration of bone morphogenic protein-7 [126], blockade of matrix metalloproteinases [127], microRNA-21 inhibition [128], endothelin-1 receptor antagonism [129], and bone marrow transplantation [130, 131]. Clinical trials are ongoing testing an anti-microRNA-21 agent (NCT02855268) and the anti-inflammatory agent bardoxolone (NCT0309185) in patients with Alport syndrome. No treatments are currently available to ameliorate the progression of hearing loss in patients with Alport syndrome.

Restoration of the GBM in patients with Alport syndrome is also a potential strategy for treatment or even potential cure. Gene-editing technologies such as CRISPR-Cas9 or exon-skipping therapies may provide additional options for patients in the future [40, 132, 133]. Protein replacement strategies could also be employed to directly target recombinant type IV collagen to the GBM. This approach has been successfully utilized in a mouse model of Pierson syndrome where injection with recombinant laminin 521 delayed onset of proteinuria [134]. Finally, chemical chaperone-based therapy was shown to enhance deposition of COL4A5 in a preclinical study [135].

Transplantation

Patients with Alport syndrome typically do well after kidney transplantation with similar or better graft survival than matched controls [136, 137] Two issues require the special attention of transplant physicians involved in the care of patients with Alport syndrome. First, evaluation of potential related donors must identify affected individuals who may have heterozygous mutations in type IV collagen genes who may be at risk for progressive CKD. A case series of women with Alport syndrome who donated their kidney showed that new-onset hypertension, proteinuria, and GFR decline were common after donation [138]. Second, posttransplant monitoring must allow early diagnosis of posttransplant anti-GBM nephritis, a complication of transplantation that is unique to Alport syndrome.

Anti-GBM nephritis occurs in 1–3% of men with X-linked Alport syndrome after transplantation [139]. The onset of anti-GBM nephritis typically occurs during the first year after transplantation and usually results in irreversible graft failure within weeks to months of diagnosis. There is a high rate of recurrence in subsequent allografts. In XLAS males, the primary target of anti-GBM antibodies is the α5(IV) chain [140, 141]. Females with XLAS who require transplantation are at little or no risk of developing anti-GBM nephritis. However, both males and females with ARAS can develop anti-GBM nephritis after transplantation. The α3(IV) chain is the primary target of anti-GBM antibodies in ARAS patients [140, 142]. Although both Goodpasture autoantibodies and anti-GBM antibodies from transplanted Alport patients target the carboxyterminal noncollagenous (NC1) domain of the α3(IV) chain, they target separate and distinct epitopes [143].

Kidney transplant biopsy with routine immunofluorescence should be performed early in the evaluation of Alport patients who develop hematuria or increased creatinine after transplantation or if circulating anti-GBM antibodies are detected. Anti-GBM nephritis after kidney transplantation has been treated with cytotoxic therapy and plasmapheresis, although such therapy has been unsuccessful in the majority of reported patients.

Pierson Syndrome

The association of congenital nephrotic syndrome with eye abnormalities in siblings was first described by Pierson in 1963 [144]. This condition is inherited in an autosomal recessive manner and accounts for ~2.5% of congenital and infantile nephrotic syndrome.

Genetics

The gene for Pierson syndrome was mapped to chromosome 3p14–p22 and identified as *LAMB2*, the gene encoding the laminin β2 chain, by Zenker and colleagues in 2004 [46]. Subsequent reports indicate that *LAMB2* mutations may be associated with isolated congenital nephrotic syndrome and with mild variants of Pierson syndrome [145, 146]. The absence of the laminin β2 chain in transgenic mice results in massive proteinuria along with retinal and neuromuscular abnormalities [147]. In these mice, proteinuria precedes the appearance of podocyte abnormalities, suggesting that the GBM contributes to the barrier function of the glomerular capillary wall. Genotype influences the phenotype in individuals with *LAMB2* mutations. Truncating or splice site mutations are more likely to be associated with complete Pierson syndrome, whereas missense mutations or small deletions are more likely to be associated with the absence of neurologic abnormalities and later onset of kidney disease [146, 148].

Clinical Features

In the earliest reports of Pierson syndrome, affected infants died within the first year of life with ESKD. Subsequent reports of additional patients described variable abnormalities on fetal ultrasound, including kidney enlargement and hyperechogenicity, oligohydramnios, placental enlargement, and/or pulmonary hypoplasia [46, 149]. Infants surviving to term exhibited congenital nephrotic syndrome accompanied by ocular globe enlargement (buphthalmos) with reduction in the size and reactivity of pupils (microcoria). Additional ocular findings in some patients included cataract, posterior rupture of the lens capsule, and retinal abnormalities. Some affected children have also been found to have muscular hypotonia, central nervous system hemangiomas, and genital abnormalities [150, 151].

Pathology

Children with Pierson syndrome demonstrate kidney biopsy findings of diffuse mesangial sclerosis and diffuse GBM abnormalities [152]. The GBM demonstrates irregular thick and thin zones on both sides of the lamina densa and may have lamellation and corrugation.

MYH9-Related Disorders

MYH9-related disorders are rare autosomal dominant conditions which may have similar clinical features as Alport syndrome including hematuria, progressive CKD, and sensorineural deafness [153]. Historically, four separate syndromes with variable phenotypes were identified that were all found to be caused by mutations in *MYH9*: Epstein syndrome, Fechtner syndrome, Sebastian syndrome, and May-Hegglin anomaly. Given the wide variability in phenotype in patients with *MYH9* mutations that do not always fit neatly into these syndromic categories, the term *MYH9*-related disease is preferred.

Genetics

MYH9 encodes nonmuscle myosin heavy chain IIA (NMMHC-IIA). NMMHC-IIA is expressed in podocytes [154], but the mechanism by which mutations in this protein might adversely affect podocyte function is unknown. It is hypothesized that *MYH9* mutations may disrupt the ability of the podocyte to produce and incorporate extracellular matrix proteins into the GBM [155]. Members of the same family may have different phenotypes.

Clinical Features

Kidney involvement occurs in about 30% of patients and consists of proteinuria with or without hematuria and progressive CKD. Most patients with *MYH9* mutations have thrombocytopenia and giant platelets which may lead to clinical bleeding disorders and limit the ability to perform kidney biopsy. In addition, the mutated NMMHC-IIA forms aggregates in leukocytes that are visible as Döhle-like bodies. Sensorineural hearing loss may present between the 1st and 6th decades of life [156], and early-onset cataracts can also be seen.

Pathology

In some patients with *MYH9*-related disease, ultrastructural changes in the GBM resemble those of Alport syndrome [157, 158]. EM may demonstrate glomerular GBM thickening, splitting, or attenuation [155]. However, glomerular expression of type IV collagen α3, α4, and α5 chains is normal in these patients [158]. Light microscopy may show mesangial proliferation, segmental glomerulosclerosis, and tubulointerstitial disease [155].

Treatment

Case series have described a reduction in proteinuria in patients with *MYH9* mutations treated with renin-angiotensin system blockade [159, 160].

Nail-Patella Syndrome

Nail-patella syndrome (NPS) is a rare autosomal dominant disorder characterized by dystrophic nails, hypoplasia or absence of the patellae, dysplasia of the elbows and iliac horns, and kidney disease [161, 162]. Affected individuals may also exhibit glaucoma and sensorineural deafness [163, 164]. The disorder affects about 1 in 50,000 individuals. Kidney involvement occurs in 30–40% of patients.

Genetics

Heterozygous mutations in the LIM homeodomain transcription factor gene *LMX1B* cause NPS [165, 166]. Mutations in the homeodomain of LMX1B protein and female sex may confer an increased risk of kidney involvement [164].

LMX1B protein is specifically expressed in glomerular podocytes, beginning at the S-shaped body stage of glomerular development [167]. Lmx1b-null mice exhibit marked reduction in GBM expression of the α3 and α4 chains of type IV collagen and the slit diaphragm proteins podocin and CD2AP [168–170]. However, changes in expression of these putative targets of LMX1B were not observed in the kidneys of patients with NPS nephropathy [171]. LMX1B was found to be essential for the maintenance of differentiated podocytes in adult kidneys through effects on actin cytoskeletal organization [172]. Mutations in *LMX1B* have also been described in patients with autosomal dominant focal segmental glomerulosclerosis without extrarenal involvement [173].

Clinical Features

Kidney. Less than half of NPS patients have clinically apparent kidney disease [174]. Symptoms including mild proteinuria with or without microscopic hematuria typically appear in adolescence or young adulthood. Occasional patients develop nephrotic syndrome and hypertension. The nephropathy is typically mild, with about a 5–10% risk of progression to ESKD. Marked differences in the severity of the nephropathy can be observed in related patients, suggesting the influence of superimposed factors. As with other glomerular disorders, proteinuria appears to be a risk factor for progression [175].

Nails. Nail abnormalities occur in over 90% of patients and are usually apparent from birth [176]. Fingernails, especially on the thumb and index finger, are more severely affected than toenails. The nails may be absent or dystrophic with discoloration, koilonychias, longitudinal ridges, or triangular lunulae.

Skeletal Defects. Over 90% of NPS patients exhibit aplasia or hypoplasia of the patellae [176]. Associated symptoms may include knee pain, effusions, dislocations, and osteoarthritis. Dysplasia of the elbows occurs in over 90% of patients [176]. Anomalies such as hypoplasia of the radial head with dislocation, posterior processes at the distal ends of the humeri, hypoplasia of the olecranon, and elongation of the neck of the radius may be associated with mild to severe limitation in extension, pronation, and supination of the forearm. About 80% of patients display osseous processes projecting posteriorly from the iliac wings, known as iliac horns, which are pathognomonic for NPS [176].

Pathology

The nephropathy of NPS has no specific features by light microscopy or routine immunofluorescence [177]. Focal segmental glomerulosclerosis and nonspecific deposits of IgM and C3 may be observed, depending on the severity of renal involvement. Characteristic lesions are observed by electron microscopy [178, 179]. With standard staining techniques, the GBM exhibits multiple lucencies, imparting a “moth-eaten” appearance. Staining with phosphotungstic acid reveals clusters of cross-banded collagen fibrils within these lucent areas with a periodicity of about 65 nm (Fig. 7). These collagen fibrils have been observed in NPS patients with no clinically evident renal disease, and there is no correlation between the extent of GBM changes and patient age, severity of proteinuria, or degree of kidney disease [180, 181]. Conversely, kidney involvement in patients with LMX1B mutations has been described without the classic features described above [182].

Treatment

There is no specific therapy for NPS renal disease. Kidney transplantation has been carried out successfully, and recurrence of renal disease apparently did not recur. Selection of living donors must be performed with care, since NPS is an autosomal dominant disorder.

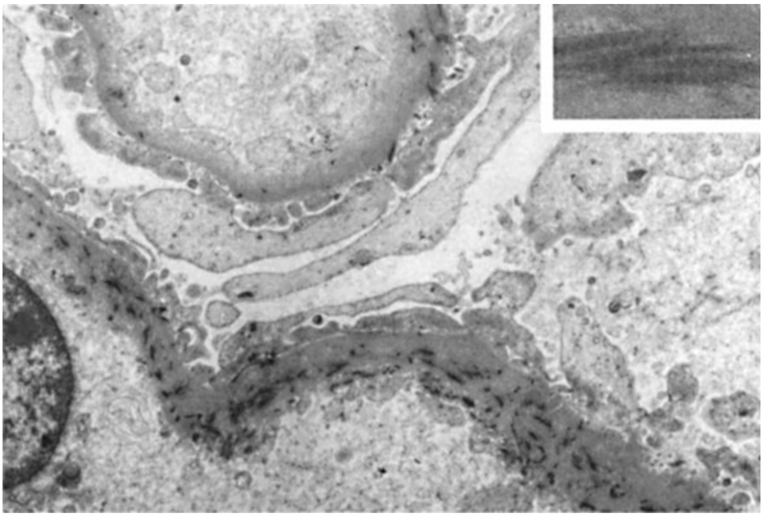

Fig. 7 Electron microscopy of nail-patella syndrome. Irregular distribution of fibrillar collagen within the GBM. Phosphotungstic acid stain (×10,500). Inset shows the typical periodicity of interstitial collagen (×48,000)

Cross-References

- ► Approach to the Child with Hematuria and/or Proteinuria
- ► Clinical Aspects of Genetic Forms of Nephrotic Syndrome
- ► Congenital Nephrotic Syndrome
- ► Technical Aspects of Renal Pathology

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Steroid-Sensitive Nephrotic Syndrome

14

Kazumoto Iijima, Agnieszka Swiatecka-Urban, Patrick Niaudet, and Arvind Bagga

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Abstract

Nephrotic syndrome is among the most common chronic kidney diseases of childhood. The condition is primary or idiopathic in more than 90% cases. Therapy with prednisone results in complete remission of proteinuria in ~90% patients, termed steroid-sensitive nephrotic syndrome (SSNS). Approximately, 70% patients with SSNS relapse, and about 50% show frequent relapses or steroid dependence that requires careful therapy with steroid sparing agents. The aim of this therapy is to prevent complications of the disease and minimize the side effects of corticosteroids and other medications. SSNS is a self-limiting illness with majority of patients outgrowing the disease by puberty. However, a small but significant proportion of patients show persistence of the illness into adulthood.

Keywords

Minimal change disease · Levamisole · Cyclophosphamide · Calcineurin inhibitors · Mycophenolate mofetil · Rituximab

Introduction

Nephrotic syndrome, characterized by edema, heavy proteinuria (>1 g/m^2/day; >40 mg/m^2/h; spot urine protein/creatinine ratio >200 mg/mmol), and hypoalbuminemia (serum albumin <3 g/dL), is among the most common chronic kidney diseases of childhood [1]. The condition has an annual incidence ranging from 1.2 to 16.9 per 100,000 children. While nephrotic syndrome is usually primary or idiopathic, evaluation may reveal an underlying systemic illness in 5–10% patients. Therapy with prednisone results in complete remission of proteinuria in 85–90% patients, termed steroid-sensitive nephrotic syndrome (SSNS). While approximately 50% patients with SSNS have frequent relapses or steroid dependence, and 3–10% show late steroid resistance, their long-term outcome is satisfactory. The prognosis is less favorable in those that fail to respond to corticosteroid treatment (steroid resistant). This chapter discusses the evaluation, management, and outcomes of patients with SSNS.

Historical Background

Hippocrates connected dropsy (edema) with kidney disease almost 2500 years ago and noticed that urine produced by people with dropsy showed bubbles [2]. In the fifteenth century, Cornelius Roelans regarded swelling of the child's whole body as one of 52 distinguished childhood diseases. In 1722, Theodore Zwinger III (Basel) made a detailed and accurate description of the signs and symptoms of nephrotic syndrome in children with hydrops (whole-body edema) and linked the condition to a problem in the kidney tubules. Measuring urine protein became available in the eighteenth century. Domenico Cotugno connected proteinuria and edema, and in 1827, Richard Bright described a triad of edema, proteinuria, and kidney disease. In the nineteenth century, histopathological examination was made in postmortem kidney specimens from patients with syphilis, who most likely had membranous nephropathy. Microscopic techniques of that period did not identify a glomerular lesion in such specimen, and physicians assumed that the condition resulted from degenerative changes in the kidney tubules. As a result, in 1905, Friedrich von Muller used the term "nephrosis" to distinguish a noninflammatory glomerular lesion in nephrotic patients with syphilis, from the inflammatory "nephritis" in other conditions. Hence, the term "pure nephrosis" or minimal change nephropathy was established. The term "syndrome of nephrosis" or "nephrotic syndrome" was introduced in 1931 by Henry Christian and Louis Leiter, who noted similarities between primary and secondary forms of the severe proteinuric disease with edema.

The invention of the electron microscope by Max Knoll and Ernst Ruska in 1931 allowed the detection of the characteristic ultrastructural changes of the glomerular podocyte, described as fusion or effacement of the foot processes. In the 1950s and 1960s, routine use of percutaneous kidney biopsy allowed the collection of data demonstrating different histologic lesions in adults with nephrotic syndrome. By contrast, minimal change disease (MCD) was the chief histologic diagnosis in patients younger than 6-years-old treated for nephrotic syndrome at the Guy's Hospital in London before 1970 [2]. In almost all of these patients, proteinuria resolved in response to corticosteroids within 4 weeks. Thus, the therapeutic response to corticosteroids became the basis of the term "steroid-sensitive nephrotic syndrome."

A collaborative effort by the International Study of Kidney Disease in Children (ISKDC) and Arbeitsgemeinschaft fur Padiatrische Nephrologie (APN) in 1960–1970 established the therapeutic approach to childhood nephrotic syndrome [3–7]. Studies were conducted, between 1967 and 1974, at 24 centers in North America, Europe, and Asia that enrolled patients from 12-weeks to 16 years of age. All patients had percutaneous kidney biopsy before therapy. Of the 521 patients, 76.6% had MCD, 7.5% had membranoproliferative glomerulonephritis (MPGN), and 6.9% had focal segmental glomerular sclerosis (FSGS). Patients were treated with prednisone 60 mg/m^2 (maximum 80 mg) daily for 4 weeks, followed by 40 mg/m^2 daily on 3 consecutive days out of seven for 4 weeks. They concluded that therapeutic response to steroids was useful for initial evaluation and therapy, because most patients had MCD and most were sensitive to corticosteroids. In contrast, patients with non-minimal lesions (MPGN or FSGS) were more commonly resistant to corticosteroids. Hence, response to steroids is widely adopted for the initial management and prevents unnecessary kidney biopsies in most children with nephrotic syndrome [8, 9].

Furthermore, the prediction that a patient who fails to respond has a glomerular lesion other than MCD would be incorrect in about one-fourth of all children with nephrotic syndrome, and as many as one-half of patients ≤6 years of age [7]. For these patients, a pathologic diagnosis is recommended. Since the 1980s, many centers have reported increased frequency of FSGS in children of different ethnicities or primary corticosteroid resistance [10–16]. Because the single-center studies used different corticosteroid regimens and FSGS can be indistinguishable from MCD during the early stage, it is not easy to compare the results of these reports with the results of the ISKDC studies [17]. Nevertheless, therapeutic response to corticosteroids remains a valid approach for the initial management of children with nephrotic syndrome [18].

Etiology

Nephrotic syndrome is caused by a glomerular injury that alters the glomerular filtration barrier and leads to urinary loss of plasma proteins.

Mechanisms of Proteinuria

Proteinuria in nephrotic syndrome results from increased passage of proteins across the glomerular capillary wall that exceeds the proximal tubular epithelial cells' absorptive capacity. Many discoveries have improved the understanding of how a normal glomerular capillary wall restricts plasma proteins from entering into the Bowman's space. It is generally accepted that the molecular size, electrical charge, and steric configuration affect the transglomerular passage of proteins.

The glomerular capillary wall comprises the fenestrated endothelial cells, glomerular basement membrane (GBM), and the dynamic structure between interdigitated podocyte foot processes, called the glomerular slit diaphragm (SD). The endothelial cells contain an anionic glycocalyx on the cell surface and form a fenestrated layer on the luminal side of the GBM. Despite the large pore size of the fenestrae (375–400 Angstrom (Å)), the endothelial cells can form an electrostatic barrier for negatively charged proteins, including albumin having a radius of 36 Å [19, 20]. Recent studies in animal models have confirmed the importance of endothelial cells and the glycocalyx in the selectivity of the filtration barrier [21, 22]. Another line of evidence for the role of endothelial cells in the filtration barrier function comes from animal studies showing that inhibition of vascular endothelial growth factor (VEGF) leads to endothelial cell dysfunction and proteinuria [23, 24].

The GBM is an acellular network formed by a scaffold of cross-linked collagen IV, laminins, and proteoglycans. GBM plays a central role in the size- and charge-selectivity with a variable contribution of each component. Type IV collagen provides mainly structural support to the glomerular capillary wall and contributes relatively less to the selectivity of the filtration barrier. This conclusion was derived from the usually mild proteinuria observed in patients with Alport syndrome [25]. Proteoglycans contain negatively charged glycosaminoglycan side chains. Heparan sulfate proteoglycans (HSPG) are responsible for the anionic charge of the GBM, and their role in the filtration barrier is widely accepted [26]. However, recent studies in murine models have questioned the role of HSPG [27–29]. By contrast, laminins were shown to play an essential role in the GBM organization and the filtration selectivity. Laminins are heterotrimeric proteins that self-assemble into a network. Animal studies show that laminin-521 is crucial for a filtration barrier [30–32]. Mutations in *LAMB2* cause Pierson syndrome, characterized by congenital nephrotic syndrome and ocular abnormalities [33].

The final filtration barrier with a functional size-selectivity of 40–45 Å resides at the glomerular SD and the podocyte–GBM interface. Podocytes are anchored to the GBM by transmembrane proteins, including integrins and tetraspanins [19]. The most abundant in podocytes, integrin α3β1, interacts with the GBM laminin-521 providing a vertical force for podocyte adhesion. Tetraspanins interact with integrins in specialized cell membrane microdomains. The interaction between tetraspanin CD151 and integrin α3β1 provides a horizontal force strengthening the podocyte adhesion to GBM. In addition to transmembrane proteins, other cellular adaptors, including integrin-linked kinase, regulate the filtration barrier at the podocyte–GBM interface. Knockout of the above proteins in animal models disrupts the podocyte–GBM interface and leads to podocyte detachment and proteinuria. These studies confirm that podocyte dysfunction plays a central role in the development of proteinuria in nephrotic syndrome.

The glomerular SD is a highly specialized junctional complex with some features of other type junctions and some unique properties. It is formed by the transmembrane protein networks, their intracellular adaptors and scaffolding proteins, and the noncontractile actin fibers that interconnect with contractile actin cables within podocyte cell bodies and major processes [19, 34, 35]. The SD contains the adherens junction components P-cadherin, β-catenin, and FAT, the tight junction members ZO-1, JAM-A, occludin, and cingulin, as well as proteins expressed in the podocytes, including nephrin, nephrin-like (neph) 1, and podocin [36–40]. The unique composition of the SD is central to its function, and loss of its specificity ultimately leads to proteinuria [41]. The characteristics of the glomerular SD molecular sieve are defined by dynamic protein–protein interactions, membrane trafficking, and signaling in the inside-out and outside-in directions [20, 42]. Understanding of the regulation of SD function is still incomplete, and new data are added each year [43].

Mechanisms of Glomerular Injury

The etiology of SSNS is unknown. It is suggested that SSNS may result from activation of the immune system in response to an infection [44]. The clinical observation that manifestations of SSNS frequently follow an upper respiratory infection seems to support such a conclusion. Clinical observations that patients initially responsive to corticosteroids may become resistant, while those with initial resistance may later show partial response suggest that steroid sensitivity, dependency and resistance represent a spectrum of a single disease [45]. It seems that the classification of nephrotic syndrome based on the response to corticosteroids, while useful therapeutically, is not etiologic and does not inform about the mechanism of glomerular injury. The presence of circulating factor(s) responsible for proteinuria has been hypothesized, but none of the candidate molecules have been confirmed [46].

Genetic Basis

Epidemiologic studies suggest that genetic risk have a role in the pathogenesis of SSNS. For example, 3% of children with SSNS have a first-degree relative with the same disease [47]. Ethnic background affects the incidence and severity of INS, with Black and South Asian children having a higher incidence than White children [15, 48]. Black children are also more likely to show corticosteroid resistance and FSGS compared to White children [15, 49].

While Mendelian inheritance has been shown in up to 30% of cases of steroid-resistant nephrotic syndrome (SRNS), it has not been confirmed in SSNS. To date, all of the genetic variants proposed to cause Mendelian SSNS can be classified as variants of uncertain significance, and many of them are shown to occur at high frequency in the general population [50, 51]. Most of the variants reported in the literature are from small, single-family studies and have not been validated in independent larger cohorts. By contrast, familial clustering of SSNS is well recognized [52]. The polygenic pattern suggests that genetic variations, perhaps together with environmental factors, may underline the susceptibility to SSNS but are not sufficient to cause the condition. How the complex polygenic pattern affects the susceptibility to SSNS remains incompletely understood.

The vast majority of relevant genetic variants identified in complex disease lie outside coding regions [53, 54]. Many of those are found to be in areas relevant for gene regulation, such as promoters and enhancers. Additionally, complex interactions between these variants are thought to increase the risk of disease [53]. Genome-wide association studies (GWAS) using candidate gene approaches found the strongest association within the HLA genetic region, HLA-DR/DQ, specifically in and around HLA-DQA1 and HLADQB1 [44, 55]. The exome-wide association study, which was not limited to a selection of genes, identified four variants (rs1129740, rs9273349, rs1071630, and rs1140343) in the HLA-DQA1/HLA-DQB1 locus in association with SSNS in a South Asian cohort [56]. Thus, GWAS has confirmed the longstanding observation that the immune system plays a critical role in SSNS [57–61]. An increased number of risk alleles in the HLA-DR/DQ region (rs1063348) and (rs28366266) was associated with an increased SSNS risk as well as decreased age at onset of disease. Additionally, the lead variant (rs1063348) around HLA-DQB1 was associated with significantly decreased expression of HLA-DRB1, HLADRB5, and HLA-DQB1 in glomeruli of children with SSNS [61]. Additional support for the involvement of the HLA-DR/DQ region in SSNS came from a GWAS investigating a large European cohort [60]. GWAS in Japanese SSNS patients confirmed the association with HLA-DR/DQ in a different ethnicity [58, 59]. The above findings underline the central role of the immune system in SSNS, but also demonstrate a complex mechanism of SSNS.

The variants in the HLA region are frequently linked together, and the composite haplotype HLA-DQA1*02:01, HLA-DRB1*07:01, and HLADQB1*02 are associated with the strongest risk of SSNS [60, 61]. The same haplotype was identified in a group of South Asian children as a risk for SSNS [62]. Conversely, HLADQA1*01, HLA-DQA1*01:03, and HLA-DRB1*13 appear to be protective in the European cohort and also in the South Asian cohort. The relevance of these haplotypes in the pathogenesis of SSNS is highlighted by preservation across different ethnic groups. By contrast, the common risk haplotype of the European and South Asian population (HLA-DQA1*02:01; HLA DRB1*07:01; HLA-DQB1*02) was not replicated in the Japanese cohorts [58, 59]. The haplotype associated with the strongest risk for the disease was HLA-DRB1*08:02, DQB1*03:02, and the haplotype with the strongest protective association was HLA-DRB1*13:02, DQB1*06:04. These specific haplotypes are extremely rare in Europeans in contrast to the Japanese population [44, 63]. Variants outside of the HLA region have also been identified as a risk for SSNS. For example, the gene *BTLN2* (butyrophilin-like 2) plays a role in the regulatory T cell differentiation, and its missense variant is associated with SSNS in different cohorts [44, 61]. The involvement of different HLA alleles and regions outside of the HLA in different ethnic populations may be explained, at least in part, by diverse infectious triggers in different geographic regions.

Immune Mechanism

The role of the immune system in the pathogenesis of idiopathic nephrotic syndrome (INS) is poorly understood despite evidence of T-lymphocyte dysregulation, local cytokine release, and a hypothetical glomerular permeability factor. In 1974, Shaloub et al. proposed that lipoid nephrosis results from a systemic dysregulation of T-lymphocytes [64]. The authors hypothesized that a T-lymphocyte-derived systemic factor acts on the GBM and increases glomerular permeability. This hypothesis was supported by animal studies showing that supernatants of T-cell hybridoma lines produced from patients with MCD induced foot process effacement and proteinuria [65]. Since then, many studies have supported the role of T-lymphocytes in the pathogenesis of MCD [66]. Expressly, the prevalence of CD8+ T-suppressor cells was noted in an animal model of nephrotic syndrome, while type 2 T-helper (Th2) cell cytokines were elevated in patients with MCD. However, of all the Th2 cytokines, only IL-13 overexpression induced foot process effacement and proteinuria in animal models [67]. Evidence from other studies, supported by some clinical observations, indicated reduced function of regulatory T-cells [66, 68]. Thus, a complex network, rather than a single factor, may contribute to the pathogenesis of MCD.

B-lymphocytes may have a role in the pathogenesis of MCD, based on histologic and hematologic evidence, successful treatment with an anti-CD20 approach, and the involvement of B-cell-derived IL-4 in podocyte injury [64, 69, 70]. There is also evidence against the role of B-cells in the pathogenesis of MCD. First, there is little or no Ig deposition on renal biopsy in MCD. Second, the CD20+ B-cell depletion by rituximab cannot be used as evidence for B-cell dysregulation because rituximab also binds in podocytes to a potential second receptor, sphingomyelin phosphodiesterase acid-like 3b (SMPDL3B) [71]. Rituximab binding to SMPDL3B was proposed to prevent podocyte injury by maintaining an intact podocyte cytoskeleton [72]. Third, some patients undergo prolonged remission after rituximab despite reconstitution of total B-cells [73]. The reconstitution of switched memory B cells and not the total B-cell population predicted relapse in children with frequently relapsing or steroid-dependent nephrotic syndrome [74]. Targeting B-cells with CD20-depleting agents such as rituximab may affect costimulatory pathways involved in T-cell activation and modify other cell lineages, such as Th17 implicated in the pathogenesis of INS [75, 76]. Finally, relapse of INS during B-cell depletion after receiving rituximab suggests the presence of diverse pathophysiological mechanisms in different forms of nephrotic syndrome [77].

Podocyte-Specific Pathways

Response to corticosteroids is thought to result from its immunosuppressive effects, although studies suggest that glucocorticoids might also have a direct nonimmune function mediated by Kruppel-like factor 15 (KLF15), targeting podocytes [78, 79]. Preclinical studies suggesting the role of podocyte-specific factors in the pathogenesis of SSNS need validation. For example, subsequent studies failed to confirm the pathogenic role of B7–1 (CD80) in MCD or FSGS [80]. Another protein implicated in the pathogenesis of MCD, hemopexin, is a plasma protein that binds sialoglycoproteins in podocytes, leading to proteinuria, foot process effacement, and cytoskeletal rearrangement [81, 82]. Another mediator upregulated in MCD and FSGS, C-maf inducing protein (c-mip), leads to proteinuria without inflammatory lesions or cell infiltration in transgenic animals [83]. Changes in the podocyte expression of angiopoietin-like 4 induce proteinuria, foot process effacement, and dyslipidemia [84]. These studies need confirmation in animal and clinical models.

Clinical Features

Nephrotic syndrome may begin during infancy, but usually occurs between the ages of 2 and 7 years. Nephrotic range proteinuria is rarely discovered during routine urinalysis. The onset of illness is often preceded by an upper respiratory tract infection, followed by edema, which becomes clinically detectable when fluid retention exceeds 3–5% of body weight [1]. Periorbital edema, frequently misdiagnosed as allergy or as conjunctivitis, is often the initial symptom. As edema is gravity dependent, periorbital puffiness decreases during the day, while it localizes at the lower extremities. In the reclining position, it localizes on the dorsal part of the body. Edema of the scrotum, penis, or labia may be seen. Edema is soft and pitting, and easily elicited. Anasarca may develop with ascites, and pleural and pericardial effusions. Rapid formation of ascites is often associated with abdominal distension, pain, and malaise; dyspnea is rare. However, these symptoms may also be related to a complication such as peritonitis, thrombosis, or rarely, pancreatitis. Cardiovascular shock is not unusual, secondary to the sudden fall of plasma albumin, with abdominal pain and symptoms of peripheral circulatory failure with cold extremities and hypotension. Blood pressure is usually normal, but sometimes elevated.

Macroscopic hematuria is rare, observed in 1% of cases. The disease may also be revealed by a complication. Peritonitis due to *Streptococcus pneumoniae* might be the presenting feature at onset of the illness [1], although this has decreased in prevalence due to immunizations. Deep vein or arterial thromboses and pulmonary embolism may also occur during the first episode or during a relapse.

Evaluation

Urinalysis

Nephrotic range proteinuria is defined as urine protein excretion >50 mg/kg/day or 40 mg/h/m^2. The morning “spot” urine protein/creatinine ratio above 2 mg/mg (200 mg/mmol) is the most commonly reported cut off value for nephrotic range proteinuria and corresponds to a dipstick value +3 or above (Table 1). In most cases, proteinuria is highly selective, consisting of albumin and low

Table 1 Investigations in patients with steroid-sensitive nephrotic syndrome

Essential at onset
Urinalysis[a]
Complete blood counts
Blood levels of urea, creatinine, electrolytes, total protein, albumin; lipid profile
Tuberculin test
Additional evaluation, if required, at onset or relapse
Tuberculin test: Areas with transmission of tuberculosis Chest radiography: Positive tuberculin test or history of contact with tuberculosis; suspected lower respiratory tract infection
Renal ultrasonography: Planned for kidney biopsy; presence of gross hematuria; suspected renal vein thrombosis
Complete blood counts: Suspected systemic infection or hypovolemia
Blood urea, creatinine, albumin, electrolytes: Severe edema; hypovolemia/dehydration; oliguria/anuria; prolonged (>72 h) diuretic therapy
Complement C3, C4, antinuclear antibody, antistreptolysin O: Gross, persistent microscopic hematuria; sustained hypertension; suspected secondary cause (systemic lupus, IgA vasculitis, C3 glomerulopathy)
Serum transaminases; hepatitis B surface antigen; antibody against hepatitis C virus: History of jaundice or liver disease
Periodic monitoring, if relapsing illness
Blood creatinine; albumin, electrolytes

[a] Quantitative estimation of urine protein is required if nephrotic range proteinuria is uncertain

molecular weight proteins. Selectivity of proteinuria, estimated by the Cameron index, is the ratio of clearance of IgG (molecular weight 150 kDa) to transferrin (80 kDa). The index in selective proteinuria is below 0.10. Nonselective proteinuria (value >0.20) is often associated with SRNS and FSGS. However, there is a considerable overlap in results, and the test has limited value. The amount of protein excreted in the urine does not reflect the quantity of protein crossing the glomerular basement membrane, since a significant amount is reabsorbed in the proximal tubule.

Urine microscopy shows fat bodies. Hyaline casts are found in patients with nephrotic range proteinuria while granular casts may be associated with acute kidney injury. Microscopic hematuria is present in 20% of cases and has limited histopathologic or prognostic significance. Urine sodium excretion is low and associated with sodium retention and edema.

Biochemistry

The total serum protein level is markedly reduced. Characteristically, albumin concentration usually falls below 2 g/dL and is often less than 1 g/dL. Reporting serum albumin concentration depends on the methodology. The values obtained by bromocresol green colorimetry are ~5.5 g/l higher than those measured by bromocresol purple (colorimetric), capillary electrophoresis, or immunonephelometry. Serum electrophoresis shows low levels of albumin and γ-globulins, and increased α-2 and β-globulins. Blood levels of IgG are markedly decreased, IgA is slightly reduced, IgM is increased, and IgE is normal or increased. Among other proteins, fibrinogen and β-lipoproteins are increased; small molecules such as antithrombin III are lost in the urine and their concentration in plasma is decreased.

Hyperlipidemia in nephrotic syndrome results from: (i) increased hepatic synthesis of cholesterol, triglycerides, and lipoproteins in response to decreased intravascular oncotic pressure; (ii) decreased catabolism of lipoproteins due to reduced activity of lipoprotein lipase that transforms VLDL to LDL via IDL; and (iii) reduced activity of LDL receptor and increased urinary loss of HDL [85, 86]. Increased plasma concentrations of proprotein convertase subtilisin/kexin type 9 (PCSK9), which reduces hepatic uptake of LDL by downregulation of the LDL receptor, have been reported in patients with nephrotic syndrome, and might contribute to hyperlipidemia [87]. Total and LDL cholesterol are elevated, while HDL cholesterol remains unchanged or low, particularly HDL2, leading to increased ratio of LDL/HDL cholesterol [88]. Patients with severe hypoalbuminemia have increased triglycerides and VLDL. Apoproteins, apo B, apo CII, and apo CIII are increased. Levels of lipoprotein Lp(a) are elevated, contributing to cardiovascular and thrombotic complications.

These abnormalities are transient and resolve following remission.

Serum electrolytes are usually within the normal range. A low sodium level may reflect renal retention of water due to hypovolemia and the syndrome of inappropriate antidiuresis. Mild reduction of plasma sodium concentration might be due to reduced fraction of plasma water, as seen in patients with severe hyperlipidemia or paraproteinemia (pseudohyponatremia). Although serum total calcium is low as a result of hypoalbuminemia, levels of ionized calcium are normal. Serum creatinine is usually within the normal range but may be high due to intravascular volume depletion or acute kidney injury [89].

Hematology

Hemoglobin levels and hematocrit are increased in patients with plasma volume contraction. Thrombocytosis is common and may reach 5×10^8 or 10^9/l.

Renal Biopsy

A kidney biopsy is not required in a child aged 1–14 years with typical features of nephrotic syndrome, who shows complete remission of proteinuria following therapy with corticosteroids. The indications for biopsy include steroid resistance, defined as the lack of complete remission despite 6-weeks daily therapy with prednisone, macroscopic or persistent microscopic hematuria, persistently elevated creatinine levels or sustained hypertension, extrarenal signs or symptoms suggesting a systemic disease, or low C3 level suggesting glomerular disease, other than minimal change nephrotic syndrome.

Nephrotic syndrome presenting in infancy is often secondary to a genetic cause and appropriate testing is indicated. If genetic analysis is not available, experts suggest performing a kidney biopsy before initiating therapy with corticosteroids. Biopsy is not indicated in SSNS before starting treatment with a corticosteroid-sparing agent as the histopathologic features (MCD, diffuse mesangial proliferation, FSGS, or IgM nephropathy) have no impact on the choice of therapy in patients who continue to be steroid sensitive. However, a kidney biopsy may be performed in patients receiving calcineurin inhibitors for assessment of nephrotoxicity.

Light microscopy shows three morphological patterns. The most common is MCD, followed by diffuse mesangial proliferation and FSGS found in 5–7% of biopsies, each [90, 91].

Minimal Change Disease (MCD)

On light microscopy, the glomeruli show normal capillary walls and normal cellularity (Fig. 1). Swelling and vacuolation of epithelial cells (podocytes) and slight increase in mesangial matrix are often observed. Mild mesangial hypercellularity and scattered foci of tubulointerstitial lesions and [5]. Vascular changes are absent. Ultrastructural changes are always present, involving podocytes and the mesangial stalks. Podocyte foot process fusion is generalized and constant (Fig. 2). There is no direct correlation between the degree of ultrastructural injury and severity of proteinuria [92]. Other epithelial changes consist of microvillus formation and the presence of numerous protein reabsorption droplets. The glomerular basement membranes are normal with no deposits; endothelial cells are often swollen. Mesangial

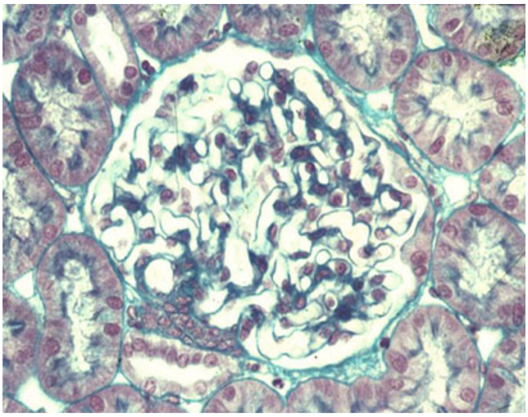

Fig. 1 Kidney biopsy specimen from a patient with minimal change disease. Normal appearance of glomeruli, tubules and interstitium (Light microscopy. Trichrome light green, ×275). (Courtesy of MC Gubler, MD)

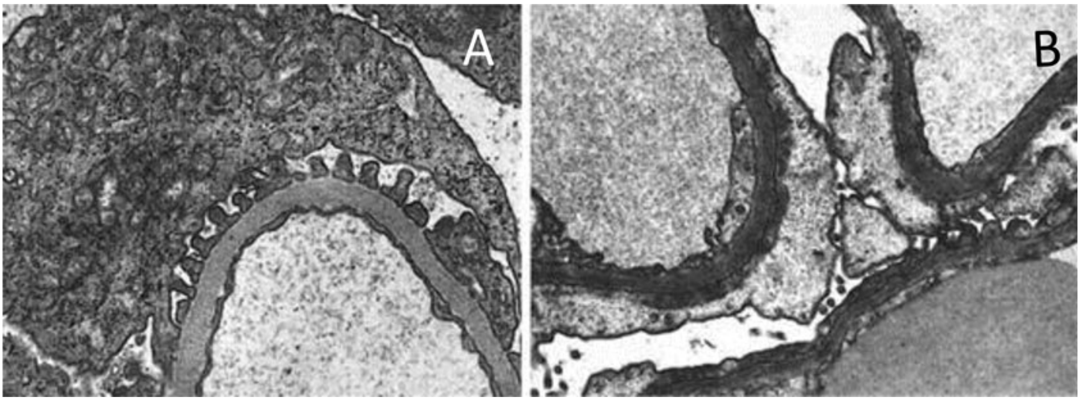

Fig. 2 Electron microscopy. A. Normal glomerular capillary loop : podocyte foot processes are attached to the regular glomerular basement membrane. B. Minimal change disease. Diffuse effacement of foot processes along the normal glomerular basement membrane. Uranyl acetate, lead citrate, ×12 000. (Courtesy of MC Gubler, MD)

alterations include mild mesangial cell hypercellularity, increased matrix, and finely granular osmiophilic deposits along the internal aspect of the basement membrane. Ultrastructural alterations are nonspecific and reverse following remission of proteinuria.

Immunofluorescence examination is negative in most cases; in some, there may be weak mesangial deposition of C3 that lacks correlation with clinical presentation or outcomes.

Diffuse Mesangial Proliferation

The biopsy shows a marked increase in mesangial matrix associated with hypercellularity, although these features are often observed in those with SRNS (Figs. 3 and 4) [5, 6, 93]. Peripheral capillary walls are normal. Electron microscopy shows foot process fusion, similar to the changes observed in MCD. The presence of mesangial hypercellularity has been found to have prognostic significance with a higher rate of progressive kidney disease in some studies [93].

Focal Segmental Glomerulosclerosis (FSGS)

Although FSGS is frequently observed in patients with SRNS, it may be observed in up to 10% of children with SSNS. FSGS affects a variable proportion of glomeruli [5, 94]. These lesions initially affect glomeruli in the juxtamedullary cortex. In order to reduce the risk of missing focal lesions, adequate sampling is necessary. It has been suggested that the risk of missing a lesion is 35% when the sample contains 10 glomeruli, and 12% if the sample contains 20 glomeruli [95].

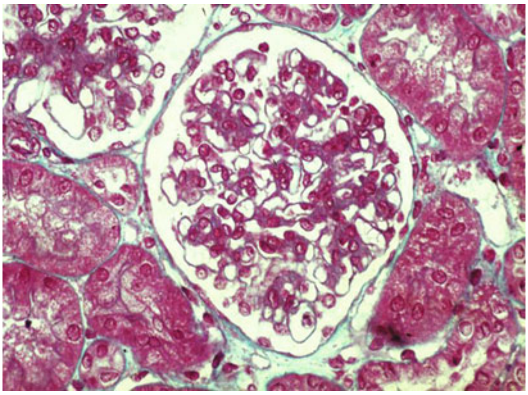

Fig. 3 Diffuse mesangial proliferation : increased number of mesangial cells and thickening of the mesangial matrix. Trichrome light green, X200. (Courtesy of MC Gubler, MD)

The focal changes are limited to a part of the tuft; other capillary loops show no modification. The lesions predominate in the deeper cortex at the corticomedullary junction [96]. Segmental lesions affect a few capillary loops, which stick together either at the hilum or at the periphery of

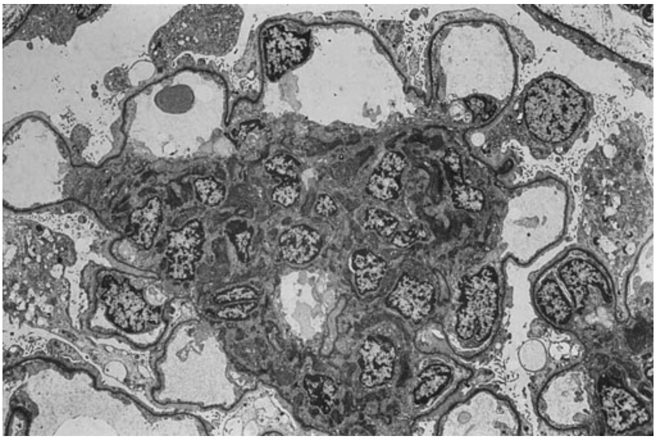

Fig. 4 Diffuse mesangial proliferation with marked increased in the number of mesangial cells, associated with diffuse effacement of podocyte foot processes. Electron microscopy. Silver methenamine ×2500. (Courtesy of MC Gubler, MD)

the tuft or at both [97, 98]. The clinical course is reported to be benign when the location of these sclerotic lesions is peripheral (tip lesions), although such findings have not been confirmed by others [96, 99]. Hyaline material is often present within the sclerotic lesions. A clear "halo" is observed at the periphery of the sclerotic segments (Fig. 5). The segmental lesion has a different aspect depending on whether it affects a group of capillary loops free in Bowman space or is adherent to Bowman capsule. The "free" sclerotic segments are always surrounded by a "crown" of flat or hypertrophied podocytes. The podocytes form a continuous layer overlying the damaged areas of the tuft and in close apposition to the clear "halo." When the sclerotic lesion is adherent to Bowman capsule, there is a direct synechiae between the collapsed capillary loops and Bowman basement membrane. The rest of the tuft and the non-sclerotic glomeruli show "minimal changes" with foot process fusion. Glomerular hypertrophy is common in FSGS, and when such hypertrophy is found in MCD, it might be predictive of progression to FSGS [100]. Tubular atrophy and interstitial fibrosis are often present and proportional to the glomerular damage [6, 101]. Focal glomerular lesions should therefore be suspected when focal tubular and interstitial changes are found associated with minimal glomerular changes.

On electron microscopy, the FSGS lesion is characterized by the presence of paramesangial and subendothelial, finely granular, osmiophilic deposits [101] with either disappearance or swelling of endothelial cells and an increase in mesangial matrix material. Fatty vacuoles may be seen, either in the middle of the abnormal deposit or in the cytoplasm of endothelial and mesangial cells. The peripheral synechiae, between podocytes and basement membrane, is formed by the apposition of acellular material in which thin and irregular layers of newly formed basement membranes are visible (Fig 6). Modifications of the podocytes consist of focal cytoplasmic degeneration, breakdown of cell membranes, and detachment of epithelial cells from basement membranes, with filling of the resulting space by cell debris and new membranes [102].

A pathologic classification of FSGS, designated the Columbia classification, is proposed with five histological variants [103]:

i. FSGS not otherwise specified (NOS) or classic FSGS, the most frequent variant.
ii. The perihilar variant with lesions located at the vascular pole of the glomerular tuft.
iii. The cellular variant with podocyte hyperplasia and endocapillary hypercellularity, foam cells, and leukocyte infiltration.
iv. The tip variant, where the lesion involves the tubular pole, is the most benign form of FSGS with presenting features and outcome more close to MCD [104].
v. Collapsing variant or collapsing glomerulopathy characterized by segmental or global wrinkling and collapse of the glomerular capillary walls with prominent hypertrophy and hyperplasia of the overlying podocytes.

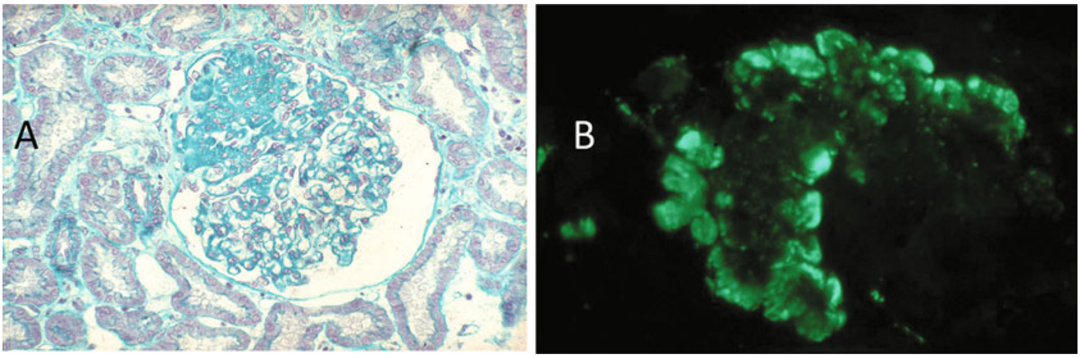

Fig. 5 Focal and segmental hyalinosis/sclerosis. A. Segmental obliteration of the glomerular tuft by hyaline deposits surrounded by a clear halo and adhesion to the Bowman's capsule. Light microscopy. Trichrome light green ×150. B. IgM deposits within a segmental lesion of hyalinosis. Immunofluorescence ×350. (Courtesy of MC Gubler, MD)

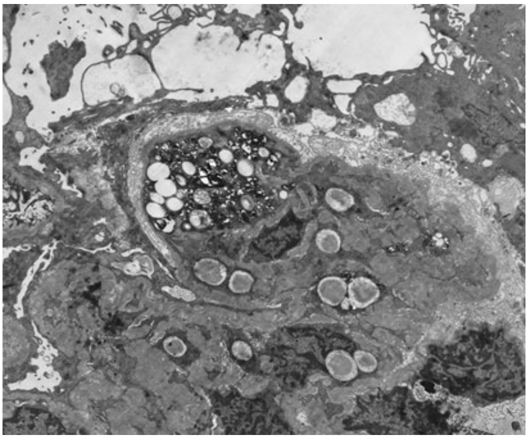

Fig. 6 Focal and segmental hyalinosis. The capillary loop is obliterated by increase in mesangial material mixed with granular deposits and lipid droplets. The lesion sis surrounded by thin layers of newly formed membranes. Podocytes are vacuolated and show diffuse loss of foot processes. Electron microscopy. Uranyl acetate and lead citrate, ×3500. (Courtesy of MC Gubler, MD)

This classification has clinical implications in terms of response to therapy and risk of progression to renal failure. For example, glomerular tip lesions have been associated with better outcomes and collapsing variant with worse outcomes [99]. The former lesion was predictive of favorable response to therapy in a pediatric series [105]. Patients with collapsing glomerulopathy have severe nephrotic syndrome, unsatisfactory response to corticosteroids, and rapidly progress to renal failure.

Immunofluorescence Patterns

IgM-Associated Nephropathy

Occasionally, patients with MCD have biopsies that show mesangial deposits of IgM, IgG, C3, and rarely IgA on immunofluorescence microscopy. Mesangial deposits of IgM are the most common (Fig. 7). Habib et al. reported on 54 children with INS and glomerular IgM deposits in the glomeruli. There was no correlation between IgM deposits and the initial response to corticosteroids or the final outcome [106]. The impact of mesangial IgM deposits on the clinical course is unclear. Some experts suggest that IgM nephropathy is a distinct entity associated with hypertension, CKD, and steroid dependence or resistance [107]. Others show that mesangial IgM deposits do not correlate with outcomes in patients with nephrotic syndrome [108].

C1q Nephropathy

This entity, first described by Jennette and Hipp in 1985, presents with mesangial deposition of immunoglobulins and complement components, predominantly C1q, and mesangial electron-dense deposits [109, 110]. Clinical features vary from asymptomatic proteinuria or hematuria to

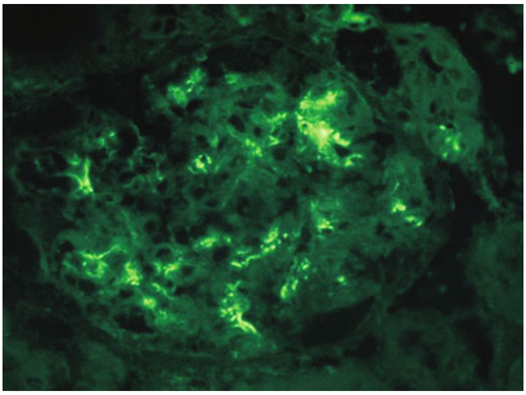

Fig. 7 Presence of mesangial IgM deposits. (Immunofluorescence ×280) (Courtesy of MC Gubler, MD)

nephrotic or nephritic syndrome, and no clinical or serologic evidence of systemic lupus erythematosus. C1q deposits may be found in association with MCD or FSGS [111]. The disappearance of C1q deposits or development of FSGS has been reported on follow-up biopsies [112]. C1q deposits in patients with MCD may be associated with frequent relapses and shorter relapse-free periods, corticosteroid dependence, and need for second-line therapy, but without impact on progression or long-term outcomes [113, 114].

IgA Deposits and MCD

Patients with SSNS may occasionally show mesangial deposits of IgA. While some experts consider the histology as being compatible with IgA nephropathy, others propose that the presence of mesangial IgA in patients with minimal change disease, i.e., without mesangial proliferation is coincidental [108, 115, 116]. These patients show rapid response to therapy with prednisolone, which is not seen in IgA nephropathy.

Therapy

Management of Initial Episode

Following the diagnosis of nephrotic syndrome, specific therapy is initiated with oral corticosteroids (prednisone or prednisolone; hereafter referred to as prednisone). In 1981, the ISKDC empirically proposed that the first episode of nephrotic syndrome be treated with daily prednisone for 4 weeks, followed by intermittent therapy (3 consecutive days in a week) for next 4 weeks, and then discontinued [7]. Later, a randomized controlled trial (RCT) showed that therapy with prednisone for 6 weeks daily, followed by alternate day for next 6 weeks was better in terms of reduced incidence of relapses over the next 12–24 months [117]. In efforts to define optimal therapy for the initial episode, several RCTs investigated the duration and dose of prednisone, based on which a 2007 meta-analysis concluded that therapy for 6 months was associated with reduced risk of relapses and lower risk of frequent relapses (relative risk, RR 0.55; 95% CI 0.39–0.80) [118]. However, most studies included in the meta-analysis had methodological flaws, resulting in high risk of bias.

Four multicenter RCTs published in the last 7 years have challenged the previous results. These studies, representing outcomes in over 800 patients across the Netherlands, the UK, Japan, and India, show that extending initial therapy beyond 8–12 weeks does not influence the time to first relapse or the risk of frequent relapses at 1–2 years' follow-up. All these studies had low risk of bias; three were placebo-controlled. A meta-analysis that included these studies showed that the risk of frequent relapses at 1–2 years follow-up was not different for 3 months or longer versus 2 months therapy (RR 0.86; 95% CI 0.65–1.13), and for 5 months or longer versus 3 months therapy (RR 0.70; 95% CI 0.45–1.08) [119–121]. Subgroup analysis, limited to studies at low risk of bias, indicated identical risk for frequent relapses in patients treated for 2–3 months versus 3–6 months. *Post-hoc* analyses of two studies suggest a trend for benefit with prolonged therapy in young children; this finding requires confirmation [120, 121].

Based on the above, experts recommend that therapy for the initial episode comprise of prednisone at a dose of 60 mg/m^2/day (2 mg/kg/day, maximum 60 mg in 1–2 divided doses) for 4–6 weeks, followed by 40 mg/m^2 (1.5 mg/kg, maximum 40 mg as single morning dose) on alternate days for the next 4–6 weeks, and then discontinued [120, 121].

Based on pharmacokinetics and variations by age, prednisone is usually dosed by body surface area in children [122]. However, estimation of body surface area involves complex formulae with variable results [123]. Calculation using body weight is more convenient but results in relative underdosing, particularly in young children [123, 124]. Underdosing, using weight-based calculations, may be associated with increased risk of relapses in some [125, 126], but not all studies [127, 128]. For young children, experts therefore prefer to administer prednisone based on body surface area [122].

Daily prednisone is administered in single or divided doses, with similar time to remission [120, 129]. Most evidence on corticosteroid therapy is limited to the use of prednisone or its biologically active metabolite, prednisolone [130]. The use of deflazacort, based on one RCT and few case series, continues to be limited [131]. Prednisone is best given following food; therapy with antacids, ranitidine, or proton pump inhibitors is not routinely required [120].

Disease Course and Therapy of Relapses

While the disease course varies in patients with SSNS, more than 75% patients show one or more relapses on follow-up [9, 132] (Table 2). Almost one-half of the relapses are precipitated by minor infections, usually of the upper respiratory tract. A relapse has conventionally, albeit empirically, been treated with prednisone at 60 mg/m^2/day (2 mg/kg/day; maximum 60 mg) in single or divided doses until remission (protein trace/nil for 3 consecutive days), followed by 40 mg/m^2 (1.5 mg/kg, maximum 40 mg) on alternate days for 4 weeks [120, 121]. Remission is achieved by 7–10 days and daily therapy is seldom necessary beyond 2 weeks. In case of persistent proteinuria, daily therapy with prednisone may be extended, to maximum of 6 weeks. Lack of remission despite treatment with 6-weeks' daily prednisone indicates late (secondary) steroid resistance that requires specific evaluation and management [121].

Retrospective studies and small RCTs suggest that reduced dose [133] or abbreviated duration of therapy [134] with prednisone is effective in

Table 2 Definitions of disease course in nephrotic syndrome

Nephrotic range proteinuria	Urine dipstick protein 3+ (300 mg/dL)[a] or more; first-morning spot urine protein to creatinine ratio (UPCR) >2 mg/mg; 24-h protein >1000 mg/m^2
Remission	Dipstick protein nil or trace (<30 mg/dL) for 3 consecutive days; UPCR <0.2 mg/mg (<0.5 mg/mg in children younger than 2-year-old); urine protein <4 mg/m^2/h
Relapse	Urine dipstick protein ≥3+ (300 mg/dL) for 3 consecutive days; UPCR >2 mg/mg, or urine protein >40 mg/m^2/h
Steroid-sensitive nephrotic syndrome	Remission within 6 weeks of therapy with prednisone at standard dose (60 mg/m^2/day or 2 mg/kg/day; maximum 60 mg/day)
Steroid-resistant nephrotic syndrome	Lack of remission despite therapy with daily prednisone at a dose of 2 mg/kg (or 60 mg/m^2) daily for 6 weeks
Frequently relapsing nephrotic syndrome	Two or more relapses in the first 6 months after stopping initial therapy, or ≥3 relapses in 1 year
Steroid-dependent nephrotic syndrome	Two consecutive relapses when on alternate day steroids or within 14 days of cessation of therapy
Significant corticosteroid toxicity	Hyperglycemia (fasting glucose >100 mg/dL, postprandial >140 mg/dL, HbA1c >5.7%); obesity (BMI > 27 kg/m^2); short stature (height < ¯2 SDS) with height velocity (<¯3 SDS for age); glaucoma; cataract(s); myopathy; osteonecrosis; or psychosis

[a] False positive if urine pH >7.0; false negative if specific gravity ≤1010

inducing and maintaining remission. Well-powered studies are required to confirm these findings and evaluate the optimal dose and duration of prednisone for treatment of relapses.

Management of Frequent Relapses

The disease course varies in patients with SSNS; 35–40% have a single episode or infrequent relapses, and 50–55% show frequent relapses (FRNS) or steroid dependence (SDNS) [9, 132] (Table 2). Risk factors for frequent relapses and steroid dependence include an early age at onset (≤3-year), delayed time to remission, and inadequate initial steroid therapy. Patients with a fast relapse (duration of initial remission less than 6 months) are also at risk for subsequent frequent relapses [9, 132, 135, 136]. Relapses may be associated with significant complications, including life-threatening infections, hypovolemia, and thrombosis. Therapy with multiple courses of high-dose prednisone is associated with significant toxicity, including obesity, cushingoid features, behavioral problems, cataract, glaucoma, glucose intolerance, hypertension, osteoporosis, and avascular hip necrosis.

Choice of Therapy

Patients with frequent relapses or steroid dependence are initially treated with long-term alternate day steroids. Those continuing to show frequent relapses or features of steroid toxicity are offered therapy with steroid-sparing agents. Since few prospective studies have compared the relative efficacy and safety of steroid-sparing agents, most guidelines do not specify the precise order or specific choice of alternative therapy [120, 121] (Table 3). The choice of steroid-sparing therapy for these patients is therefore based on considerations of its efficacy and adverse effects, patient age, steroid threshold, severity of relapses, and features of steroid toxicity. Potent medications are preferred in patients with high steroid threshold (>1 mg/kg on alternate days) relapses, relapses associated with life-threatening complications, or if associated with significant steroid toxicity (Table 2). Figure 8 summarizes a proposed schema for the management of these patients. Patients who relapse despite therapy with two or more steroid-sparing agents (difficult-to-treat steroid-sensitive disease) are considered for therapy with calcineurin inhibitors, and failing that rituximab. While the approach to management indicated in Fig. 8 suffices in most instances, individual situations may require different preference. Therapy with oral cyclophosphamide is avoided in young patients, and in pubertal or postpubertal boys. Therapy with calcineurin inhibitors may be preferred to mycophenolate mofetil (MMF) in very young patients with significant steroid toxicity. The use of rituximab is avoided in young children due to the risk of hypogammaglobulinemia.

The above interventions aim to reduce the frequency of relapses, and wherever possible, stoppage of corticosteroid therapy. Occurrence of sustained remission or up to one relapse in 6 months is considered acceptable, and except in severe steroid dependence, therapy with corticosteroids may be tapered and discontinued over few months.

Long-Term Alternate Day Steroids

The dose of prednisone is tapered to the lowest dose required to maintain the patient in remission without significant adverse effects. A dose of 0.5–0.7 mg/kg given on alternate days for 6–12 months is effective in reducing relapses or maintaining remission in 30–40% patients [120]. While this regimen is commonly used as first line to reduce the risk of relapses, there are no randomized trials to support its use.

Since relapses are precipitated by minor infections, three RCTs have examined the role of short-term (5–7 days) daily administration of steroids in reducing infection-related relapses [137–139]. Although all studies showed reduced relapse rates with the intervention, one had a small sample size

Table 3 Immunosuppressive medications for frequent relapses

Medication	Dose	Duration	Adverse effects	Recommended monitoring
Prednisone	0.5–0.7 mg/kg on alternate days[a,b] Reduce dose if sustained remission	6–12 months	Cushingoid features; short stature; hypertension; raised intraocular pressure; glucose intolerance; cataract; elevated transaminases	Screen for side effects, hypertension Anthropometry q 3–6 months; eye evaluation q 6–12 months; glucose and transaminases q 3–6 months
Levamisole	2–2.5 mg/kg on alternate days	2–3 years	Leukopenia, ANCA positive vasculitic rash, high transaminases, seizures	Blood counts q 2–3 months; transaminases q 4–6 months
Cyclophosphamide	2–2.5 mg/kg/day orally	8–12 weeks	Leukopenia, alopecia, infections; discolored nails; hemorrhagic cystitis; risk of gonadal toxicity and malignancies	Blood counts q 2 weeks Maintain hydration; discontinue during infections Co-administer with prednisone 1 mg/kg AD; taper and discontinue prednisone after 4–8 weeks
Mycophenolate mofetil	600–1200 mg/m^2/day in divided doses; AUC >45 mg·h/L	2–3 years	Abdominal pain, diarrhea, nausea, weight loss; viral warts; leukopenia; elevated transaminases	Screen for adverse effects Blood counts and transaminases q 3–6 months
Cyclosporine	4–5 mg/kg/day in divided doses; trough 50–100 ng/mL[a]	2–3 years	*Both:* Nephrotoxicity, hyperkalemia, hepatotoxicity *Cyclosporine:* Gingival hyperplasia, hypertrichosis; hypertension; dyslipidemia *Tacrolimus*: Tremors, seizures, headache; diarrhea; glucose intolerance; hypomagnesemia	Screen for cosmetic side effects, tremors, diarrhea, hypertension Creatinine, potassium at 2–4 weeks, q 3–6 months Liver function tests, glucose, uric acid, magnesium and lipids q 3–6 months
Tacrolimus	0.1–0.2 mg/kg/day in divided doses; trough 4–8 ng/mL[a]	2–3 years		
Rituximab	375 mg/m^2 as slow IV infusion	Two doses one week apart[c]	Chills, fever; serum sickness; bronchospasm; acute lung injury Neutropenia; *P. jirovecii* pneumonia; reactivation of hepatitis B or JC virus; hypogammaglobulinemia	*Pre-dose*: Blood counts, transaminases; hepatitis B and HIV serology; immunoglobulin G (IgG) level *Post-therapy:* Monitor CD19 and blood counts; IgG level; consider cotrimoxazole prophylaxis

[a] May reduce dose further if remission is sustained

[b] During infections, administer alternate day prednisolone at 0.5 mg/kg every day for 5–7 days to prevent relapse

[c] One to two additional doses are given at weekly intervals if CD19+ cells are >5/μL (or > 1% of CD45+ cells) despite two doses of rituximab (Modified from Indian Pediatrics 2021)

[137], and another did not examine long-term benefits [138]. A prospective large RCT showed that daily administration of small dose of prednisone during infections independently reduced annual relapse rates by 59% (rate ratio, 0.41; 95% CI 0.3, 0.6) without the risk of steroid toxicity. Six patients needed to be treated to prevent the occurrence of frequent relapses in one [139]. Based on the above findings, expert guidelines suggest that the frequency of administration of prednisone be increased from alternate day to daily during episodes of fever or upper respiratory tract infection

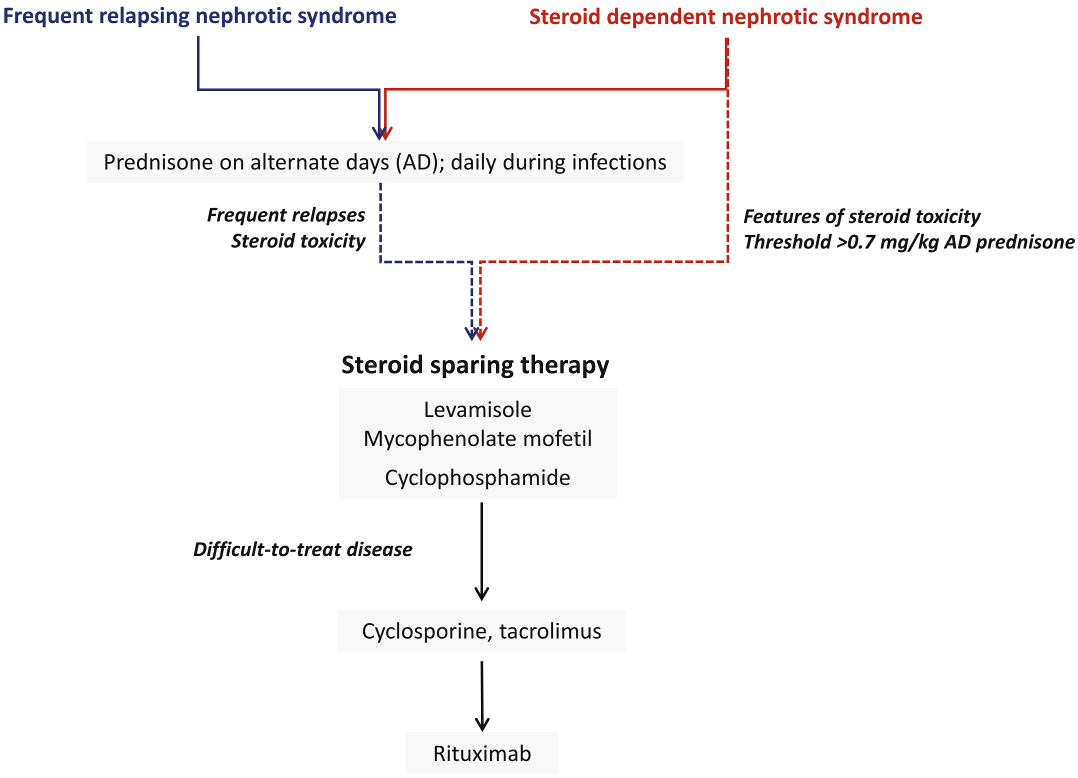
Frequent relapsing nephrotic syndrome
Steroid dependent nephrotic syndrome
Prednisone on alternate days (AD); daily during infections
Frequent relapses
Steroid toxicity
Features of steroid toxicity
Threshold >0.7 mg/kg AD prednisone
Steroid sparing therapy
Levamisole
Mycophenolate mofetil
Cyclophosphamide
Difficult-to-treat disease
Cyclosporine, tacrolimus
Rituximab

Fig. 8 Management of frequently relapsing or steroid dependent nephrotic syndrome. The initial strategy is to administer prednisone at a dose of 0.5–0.7 mg/kg on alternate days for 6–12 months. Daily therapy at the same dose for 5–7 days, during minor infections, prevents infection-associated relapses. Patients who relapse at prednisone threshold >0.7 mg/kg or show steroid toxicity require therapy with steroid-sparing medications (Table 3). Levamisole or mycophenolate mofetil (MMF) are preferred agents for mild disease. Patients with high steroid threshold (>1 mg/kg on alternate days) or with significant steroid toxicity (Table 2) may be treated with MMF at higher doses (1000–1200 mg/m^2/day) or cyclophosphamide. The use of cyclophosphamide is avoided in children <5–7 year-old and in peripubertal boys due to reduced efficacy and risk of gonadal toxicity, respectively. Patients who relapse despite therapy with two or more steroid-sparing agents are considered for therapy with calcineurin inhibitors (CNI; cyclosporine or tacrolimus) and rituximab. (Modified from Indian Pediatrics 2021)

[120, 121]. A trial comprising 365 patients (PREDNOS 2; ISRCTN10900733) failed to show benefit of 6-days low dose prednisone in reducing the risk of infection-associated relapses.

More than four decades ago, Leisti et al. proposed that suppression of the hypothalamo-pituitary-adrenal axis was in itself a risk factor for relapses, and administration of low-dose daily prednisone was effective in preventing repeated episodes of nephrotic syndrome [140]. Data from a case series [141] and an open-label RCT [142] suggests that low dose (0.2–0.3 mg/kg) daily prednisone is associated with fewer relapses than twice the dose (0.5–0.7 mg/kg) on alternate days. The strategy led to lower steroid requirement and was not associated with toxicity [142]. These findings require confirmation in studies with longer follow-up that are powered to examine adverse effects, including suppression of the hypothalamo-pituitary-adrenal axis.

Children with FRNS (or SDNS) may be treated with steroid-sparing agents when low-dose alternative-day prednisone therapy fails and/or severe adverse steroid-related events develop. A recent Cochrane systematic review of RCT indicated that cyclophosphamide, levamisole, calcineurin inhibitors, mycophenolate mofetil (MMF), and rituximab substantially reduce the incidence of relapse in children with FRNS or SDNS [143]. There are limited studies that compare the efficacy of steroid-sparing medications. The choice of the agent is chiefly based on drug efficacy, adverse effects, patient condition, and physician preference. A suggested schema for management is shown in Fig. 8. Larger studies are needed to determine how these medications should be used in patients.

Cyclophosphamide

Cyclophosphamide, the most commonly used steroid-sparing agent, has been documented in RCTs to effectively treat FRNS [144, 145]. The Cochrane review reported that cyclophosphamide significantly reduced relapse risk at 6–12 months compared to prednisone alone (4 studies, 161 children; risk ratio (RR) 0.47, 95% CI 0.34–0.66) [143]. Several reports demonstrate that cyclophosphamide is not sufficiently effective for SDNS [146–149]. A systematic review reported that remission rates in children with FRNS were 72% after 2 years and 36% after 5 years; rates in patients with SDNS were 40% and 24%, respectively [150]. Major side effects of this agent include gonadal dysfunction (azoospermia), leukopenia, infection, alopecia, hemorrhagic cystitis, and hepatic dysfunction. The risk of azoospermia is higher in boys of pubertal age (Tanner stage 2 or greater) or postpubertal age, when cumulative dose of cyclophosphamide exceeds 100–300 mg/kg, although higher doses may be safe in prepubertal boys [150, 151]. Usually, significantly lower doses of cyclophosphamide are prescribed typically 2–2.5 mg/kg for 8–12 weeks (total dose 180–200 mg/kg), with a maximum daily dose of 100 mg and only as a single course [120, 121, 152]. The risk of female infertility is considered to be lower and a cumulative dose ~200 mg/kg is reported safe, as infertility occurred at a dose of 300 mg/kg or higher [150, 151]. Approximately 32% of patients with nephrotic syndrome develop leukopenia, during cyclophosphamide therapy. The dose of cyclophosphamide is reduced if the leukocyte count drops below 4500/mm^3; therapy is suspended when the count falls below 3000/mm^3 [150].

Levamisole

Levamisole has immunomodulatory properties and a favorable side effect profile. More than 25 years ago, it was reported that levamisole reduced the risk of relapse compared with placebo [153]. However, levamisole was withdrawn from the market in 1999 in the United States because of adverse effects, mainly agranulocytosis and antineutrophil cytoplasmic antibody (ANCA)-positive vasculitis, and in Europe in 2004 because of a lack of clear indications [154]. Nevertheless, in many centers, levamisole has been used as the first-line steroid-sparing treatment of FRNS or SDNS for decades [120]. Recently, an international, multicenter, double-blind, placebo-controlled randomized trial was conducted to examine the efficacy and safety of levamisole in children with FRNS and SDNS. Between 100 days and 12 months after start of study drugs, the time to relapse (primary endpoint) was significantly increased in patients treated with levamisole compared to placebo [hazard ratio (HR) 0.22, 95% CI 0.11–0.43]. Six percent of placebo versus 26% of patients receiving levamisole were in remission at 12 months. The most frequent serious adverse event was asymptomatic neutropenia, which was reversible spontaneously or after treatment discontinuation. Thus, levamisole prolonged the time to relapse and prevented recurrence during 1-year therapy compared with placebo [155]. According to the Cochrane review, levamisole was as effective as MMF in ensuring sustained remission at 12-months (1 study, 149 children, RR 0.90, 95% CI 0.70–1.16) [143]. Similarly, levamisole was as effective as cyclophosphamide in the proportion of children relapsing at 6–9 months (2 studies, 97 children, RR 1.17, 95% CI 0.76–1.81), at 12 months (1 study, 40 children, RR 0.89, 95% CI 0.68–1.16), and at 24 months (1 study, 40 children, RR 0.89, 95% CI 0.73–1.10). Adverse effects (infection, leukopenia) may not differ between these treatment groups [143].

Cyclosporine

Cyclosporine is effective for FRNS or SDNS, although most patients experience relapses after discontinuing the drug (cyclosporine dependence) [156–163]. Blood concentration of cyclosporine should be monitored, usually by 12-h trough or C2 (2-h post-dose) levels. In a Japanese multicenter prospective RCT of cyclosporine in 44 children with FRNS, higher 2-year relapse-free rate was shown in the dose-adjustment group (12-h trough 80–100 ng/mL for 6 months, and 60–80 ng/mL for 18 months) than in the fixed-dose group (2.5 mg/kg/day for 24 months) (50% vs. 15%, $P = 0.006$) [156]. Thereafter, a multicenter study using a microemulsified preparation of cyclosporine (Neoral®) in 62 patients, conducted with dose adjustments based on the above trough levels, showed that the medication was effective and safe (2-year relapse-free rate 58%; nephrotoxicity 8.6%) [157]. Another study demonstrated that long-term cyclosporine therapy (more than 2 years; median 34 months) with trough level of 50–100 ng/ml is effective and safe [159]. The area under the time-concentration curve ($AUC_{0–4}$) of cyclosporine most strongly correlates with C2 in patients with nephrotic syndrome and in kidney transplant recipients. With this background, a multicenter RCT was conducted in Japan in 93 children with FRNS to compare two different target C2 levels: a higher C2 group (target 600–700 ng/mL for the first 6 months, followed by 450–550 ng/mL for the next 18 months) and a lower C2 group (target 450–550 ng/mL for the first 6 months, followed by 300–400 ng/mL for the next 18 months). There was no difference in the proportion of patients who were relapse-free at 24 months. However, patients in the high C2 group had fewer relapses and were likely to show infrequent relapses during the study period [164]. Results from these studies show that the dose of cyclosporine should be adjusted within target levels (trough 50–100 ng/ml; C2 300–700 ng/ml).

Chronic nephrotoxicity is the most problematic side effect of cyclosporine; its risk is increased after prolonged use (2 years or more) [165, 166]. Since it is not possible to diagnose cyclosporine-induced chronic nephrotoxicity by urinalysis and blood tests, a kidney biopsy might need to be performed in case of long-term administration; prolonged therapy is avoided [167]. Treatment with cyclosporine may result in cosmetic side effects (hypertrichosis, gingival hypertrophy), infections, hypertension, and the posterior reversible encephalopathy syndrome [156–163, 168].

Tacrolimus

Although there are no RCTs, several case series suggest that tacrolimus is effective in patients with FRNS or SDNS [169–171]. In a nonrandomized prospective study on 72 patients, tacrolimus and MMF were found to be similarly effective in maintaining remission [172]. A retrospective study also suggested that tacrolimus was more effective than MMF in prevention of relapses, but resulted in adverse events such as infections and pancreatitis [173]. Tacrolimus may therefore be considered for treating FRNS or SDNS when cyclosporine cannot be used because of cosmetic side effects (hypertrichosis, gingival hypertrophy).

A multicenter, open-label RCT to examine whether tacrolimus is non-inferior to cyclosporine in patients with FRNS or SDNS (JSKDC06; UMIN000004204) is in progress. Potential onset of diabetes mellitus and chronic nephrotoxicity are important side effects of tacrolimus; the latter often correlates with high trough levels of the agent [169]. Based on experience with kidney transplantation, trough levels in most previous studies were adjusted to 5–10 ng/mL.

Mycophenolate Mofetil (MMF)

MMF, a purine metabolism antagonist, has been used as a steroid-sparing agent for FRNS or SDNS because of its favorable side effect profile and lack of nephrotoxicity. RCTs suggest that MMF was well tolerated and beneficial in patients with FRNS or SDNS, but less effective than cyclosporine or tacrolimus [174–176]. MMF is recommended as steroid-sparing therapy in the Children's Nephrotic Syndrome Consensus

Conference [177], KDIGO [121], and the Indian Society of Pediatric Nephrology [120] guidelines, for patients with FRNS or SDNS. According to the Cochrane review, though with low certainty evidence, MMF has little or no difference compared to cyclosporine in the proportion of patients who relapse within 12 months (2 studies, 82 patients; RR 1.90, 95% CI 0.66–5.46) and relapse rate per year (3 studies, 142 patients; mean difference 0.83, 95% CI 0.33–1.33) [143]. Therapy with MMF, compared with cyclosporine, decreases the proportion of patients with hypertrichosis (3 studies, 140 patients; RR 0.23, 95% CI 0.10–0.50) and gingival hypertrophy (3 studies, 144 patients; RR 0.09, 95% CI 0.02–0.47); there were no differences in the proportion of patients with other adverse effects (hypertension, lymphopenia, decreased GFR, pneumonia, and diarrhea).

Therapy with MMF is an important step in the management of patients with FRNS or SDNS. Patients who respond to therapy with reduction in frequency of relapses may receive treatment for 1–3 years [120, 121]. Chief adverse effects include reduced blood counts, abdominal pain and diarrhea, and an increased risk of infections. Since the absorption of MMF is widely variable, it is desirable that its trough and AUC levels be monitored [174, 178]. Because MMF is teratogenic, contraceptive advice is required for females in and after puberty.

Mizoribine

Mizoribine, with action similar to MMF, has been examined in Japan. A double-blind placebo-controlled trial that compared 48 weeks' treatment with mizoribine (4 mg/kg/day) or placebo in FRNS or SDNS showed no difference in relapse rates [179]. A number of studies suggest that higher doses (6–10 mg/kg) of mizoribine might be more effective [180–184]. Hyperuricemia is an important side effect. A recent study showed sustained remission in 16 of 47 patients treated with mizoribine. They also found that C2/(single dose/body weight), Cmax/(single dose/body weight), and $AUC_{0–4}$/(single dose/body weight) for mizoribine were higher in those in sustained remission compared to those who relapsed [183]. Therapeutically effective concentrations were: Cmax $\geq$2 μg/mL, and AUC $\geq$10 μg. h/mL [184]. A RCT (JSKDC05, UMIN000005103) is in progress to examine the efficacy and safety of therapy with standard prednisone and high-dose mizoribine, to standard prednisone alone in patients with SSNS who relapse within 6 months of initial remission [185].

Rituximab

Approximately, 20% of patients with SSNS continue to show difficult-to-treat FRNS or SDNS during or after immunosuppressive therapies. RCTs and multiple case series suggest that treatment with rituximab, a chimeric anti-CD20 monoclonal antibody, was well tolerated and reduced relapse rates and enabled discontinuation of steroids and other medications, such as MMF and calcineurin inhibitors [186–190].

An open-label RCT in Italy showed that a single dose of rituximab and lower doses of prednisone and calcineurin inhibitors was non-inferior to standard therapy with calcineurin inhibitors and steroids in maintaining short-term remission [191]. A multicenter double-blind placebo-controlled trial in Japan examined the efficacy and safety of rituximab versus placebo in 48 children with complicated FRNS or SDNS [192]. The rituximab group received 375 mg/m^2 body surface area of the IV medication once weekly for 4 weeks. The control group received placebo at the same frequency. Prednisone and immunosuppressive drugs were gradually tapered, and patients were followed for 1 year. The 50% relapse-free period (267 vs. 101 days; HR 0.27; 95% CI 0.14–0.53, $P < 0.0001$) and the daily steroid dose was lower in those treated with rituximab than placebo (9.1±5.9 vs. 20.9±9.3 mg/m^2/day, $P < 0.0001$) up to 1 year. Most adverse events were mild; rate of serious adverse events and infusion reactions was similar. Based on these results, the Ministry of Health of Japan approved the use of rituximab for patients with complicated FRNS or SDNS in 2014. An open-label RCT in Korea showed similar results [193].

Follow-up of the above RCT [194] and cohorts show that most patients have recurrent relapses after recovery of peripheral B cell counts, notably switched memory B cells [74]. Further modifications, including repeated courses of rituximab or additional therapies, may be necessary for maintaining long-term remission. Studies suggest that repeat rituximab infusions after B cell recovery or four-times infusions at 3-month intervals induced long-term remission without serious adverse events in children with SDNS [188, 195]. However, the effect of persistent B-cell depletion on the developing immune system in children is unknown. Additionally, poor efficacy of vaccination under persistent B-cell depletion is a problem.

A cohort study in Japan suggested that maintenance therapy with MMF after rituximab administration was effective for maintaining long-term remission in children with complicated FRNS or SDNS [196]. A multicenter double-blind RCT (JSKDC07) is in progress to assess the efficacy and safety of MMF after rituximab treatment in children with complicated FRNS or SDNS.

The efficacy, safety, and cost-effectiveness of various dosing regimens should be compared to determine an appropriate rituximab treatment regimen for complicated FRNS or SDNS. In a retrospective cohort, the time to first relapse was significantly shorter in patients who received 1–2 doses of initial rituximab infusion compared with those who received 3–4 doses. However, the proportion of patients with long-term remission was not related to the number of initial doses [197]. An international large retrospective cohort study suggested that children with complicated FRNS or SDNS on low-dose rituximab without maintenance immunosuppression had the shortest relapse-free survival [198]. Further studies are required to determine the optimal dose and long-term safety of rituximab, and the role of maintenance therapy.

Rituximab has been used as a first-line treatment for uncomplicated SDNS in many centers in European countries. Although some RCTs indicated potential for rituximab as first-line drug for early uncomplicated FRNS or SDNS [199–201], further studies are required to clarify the efficacy and safety of this drug for uncomplicated FRNS or SDNS.

Rituximab is associated with serious adverse events, including fatal hepatitis from reactivation of hepatitis B virus [202], progressive multifocal leukoencephalopathy [203], pulmonary fibrosis [204], fulminant myocarditis [205], *Pneumocystis* pneumonia [206], ulcerative colitis [207], agranulocytosis [208], and hypogammaglobulinemia [209].

Late Steroid Resistance

Overall, 4–10% of children with SSNS may develop late (secondary) steroid resistance [11, 210, 211]. Patients with late steroid resistance often achieve complete remission with immunosuppressive therapy, such as calcineurin inhibitors [210]. Even in calcineurin inhibitor-resistant patients, significantly more patients with late resistance are reported to achieve remission with rituximab than those with initial steroid resistance [211, 212]. In contrast, patients with late steroid resistance experience significantly more recurrence (recurrent FSGS) in renal allografts than patients with initial steroid resistance [213].

Supportive Care

Nutrition

Nephrotic syndrome results in massive proteinuria that leads to hypoalbuminemia. In the past, high-protein diets were recommended to replace the protein lost in urine. On the contrary, studies in adults with decreased renal function suggest that protein restriction might be renoprotective and is therefore recommended [214]. In patients with pediatric SSNS, urine protein excretion decreases within 2 weeks after start of steroid therapy and serum albumin levels return to normal. Children with SSNS and normal renal function are advised protein consumption and caloric intake, as appropriate for healthy children of that age [120, 215].

Edema, Hypovolemia, and Fluid Overload

Mild edema can be managed with dietary sodium restriction to <2 mEq/kg/day and fluid restriction to two-thirds of maintenance. The use of diuretic agents or human albumin is these cases is not recommended. For symptomatic edema, the combination of sodium restriction, and use of diuretic agents or human albumin, based on evaluation of fluid status, are recommended. Clinical and laboratory investigations that aid determination of fluid status include estimation of weight, hematocrit, serum electrolytes, fractional excretion of sodium, urinary potassium index, chest radiography, and ultrasonography (Table 4) [120, 215, 216].

When circulatory failure with decreased effective circulating volume is observed (underfill), the use of IV crystalloid and/or human albumin is recommended. Patients showing normal or increased effective circulating volume (overfill) require treatment with diuretic agents, most commonly frusemide (Table 5). Combination therapy with human albumin and frusemide provides additive diuresis. IV albumin is administered cautiously, keeping in mind the risk of fluid overload [120]. An algorithm for treatment of edema in nephrotic syndrome is shown in Fig. 9 [120, 216].

Complications

Infections

Children with nephrotic syndrome are immunocompromised due to hypoproteinemia, low levels of IgG and urinary losses of opsonins, and immunosuppressive therapy. Before steroid therapy was introduced, the annual mortality rates for children with nephrotic syndrome were ~ 20%, attributed chiefly to severe bacterial infections. Therapy with corticosteroids induces remission and corrects hypoalbuminemia and anasarca, and was associated with reduction in infection-associated mortality. However, patients with FRNS or steroid resistance often require long-term treatment with steroids and immunosuppressive agents. Such patients remain immunocompromised and are at risk of severe systemic infections. Prompt diagnosis and management of these infections is an important management target; vaccination is advised where applicable [215].

Common sites of bacterial infections in children with SSNS include peritonitis, pneumonia, sepsis, and cellulitis [120, 216]. *Streptococcus pneumoniae* is the chief bacterial infection in patients with nephrotic syndrome; usual sites of infection with this organism include peritonitis, pneumonia, and sepsis [120, 215]. Gram negative bacilli, chiefly *Enterobacteriaceae*, are

Table 4 Features of hypovolemia during relapse of nephrotic syndrome

Clinical features
Abdominal pain, vomiting, lethargy
Prolonged capillary refill time; cold extremities
Tachycardia, low volume pulses
Low blood pressure; postural hypotension
Biochemical indices[a]
Elevated hematocrit
Fractional excretion of sodium <0.2%
Urinary potassium index [urine K^+/urine $Na^+ + K^+$] >0.6
High levels of plasma renin, aldosterone, vasopressin
Ultrasonography: Decreased inferior vena cava diameter, increased collapsibility index

$$\text{Fractional excretion of sodium} = \frac{\text{urine Na}^+ \times \text{serum creatinine}}{\text{serum Na}^+ \times \text{urine creatinine}} \times 100$$

[a] When analyzing urine electrolytes, the patient should be off diuretics, and on normal salt and water intake

Table 5 Diuretic agents for infants and children

Agent	Dose	Interval, h	Route	Dose in adults
Loop diuretics				
Furosemide	Neonates: 1 mg/kg/dose	12–14	IV, oral	40–80 mg QD daily or alternate days
	Infants/children: 1–4 mg/kg/day	6–12	Oral	
	1–2 mg/kg/dose	6–12	IV	
	Infuse at 0.1–0.4 mg/kg/h	Infusion	IV	
Thiazide diuretics				
Trichlormethiazide	Infants: 0.04 mg/kg/dose	12–24	Oral	2–8 mg/day at 1–2 doses
Hydrochlorothiazide	Infants: 1–2 mg/kg/day	12–24	Oral	25–100 mg QD or BID
Metolazone	0.2–0.4 mg/kg/day	12–24	Oral	2.5–10 mg QD
Mefruside	Infants: 15 mg/day for 3 years old	12–24	Oral	25–50 mg once (morning)
	25 mg/day for 7.5 years old			Or twice (morning, daytime)
	25–50 mg/day for 12 years old			
Aldosterone antagonists				
Spironolactone	Preterm infants (<32 weeks): 1 mg/kg/day	24	Oral	50–100 mg/day divided doses
	Mature infants: 1–2 mg/kg/day	12	Oral	
	Infants/children: 1–3 mg/kg/day	6–12	Oral	
Potassium canrenoate	Infants: 1–4 mg/kg/day	12–24	IV	100–200 mg IV once or twice daily
				Not exceed 600 mg/day
				Treatment period within 2 weeks
Triamterene	Infants: 1–2 mg/kg/day	8–12	Oral	90–200 mg/day, 2–3 doses

IV intravenous, QD once a day, BID twice a day

increasingly important in these children. Clinicians should be aware of the risks of mycobacterial infections (certain regions of the world), *P. jirovecii* pneumonia, and disseminated varicella.

Medications, such as cyclophosphamide, MMF, and calcineurin inhibitors, are being increasingly used in SDNS and resistant nephrotic syndrome. There are few published studies on the risk of systemic infections in patients treated with these agents in childhood. There is insufficient evidence for the efficacy of IV immunoglobulins (IVIG) and prophylactic antibiotics in patients with severely low levels of IgG and immunosuppressive therapy, respectively [215]. A meta-analysis suggested that rituximab was a risk factor for pneumocystis infection, and that prophylactic administration of cotrimoxazole is recommended [217].

Immunizations

Because acquired infection may lead to severe disease in patients with nephrotic syndrome, appropriate immunization is performed. Multiple expert groups have suggested immunization guidelines, which should be adhered [120, 215, 218]. Inactivated vaccines are preferred in patients receiving therapy with corticosteroids and immunosuppressive medications. There is no increased risk of relapses following these vaccines [219]. Inactivated vaccines are administered 6 months after the last dose of rituximab [218].

Live attenuated vaccines should not be administered to patients receiving corticosteroids or other immunosuppressive agents. However, the decision to use attenuated vaccines may be determined on a case-by-case basis. A study in

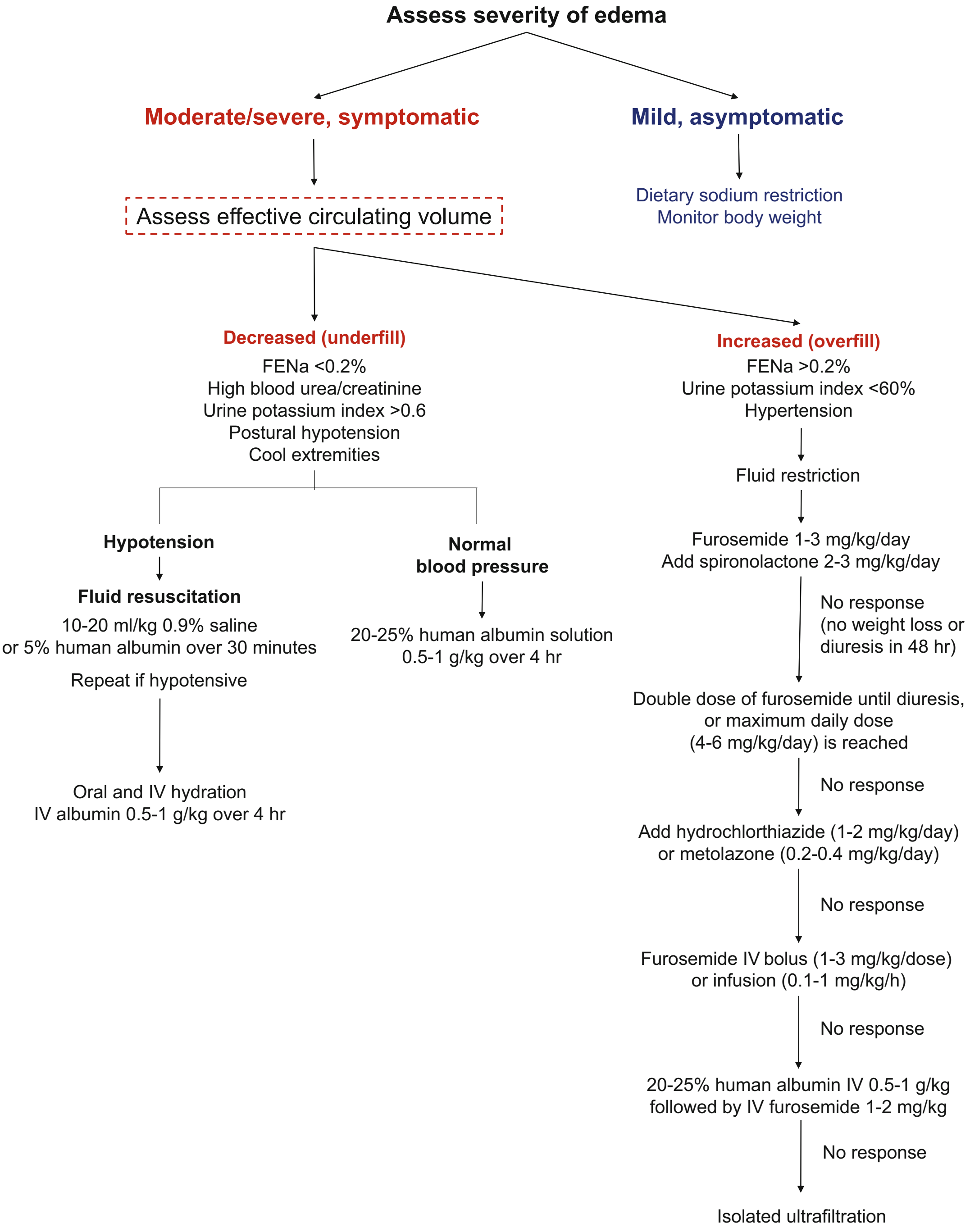
Assess severity of edema
Moderate/severe, symptomatic
Mild, asymptomatic
Assess effective circulating volume
Dietary sodium restriction
Monitor body weight
Decreased (underfill)
FENa <0.2%
High blood urea/creatinine
Urine potassium index >0.6
Postural hypotension
Cool extremities
Increased (overfill)
FENa >0.2%
Urine potassium index <60%
Hypertension
Fluid restriction
Hypotension
Fluid resuscitation
10-20 ml/kg 0.9% saline
or 5% human albumin over 30 minutes
Repeat if hypotensive
Oral and IV hydration
IV albumin 0.5-1 g/kg over 4 hr
Normal
blood pressure
20-25% human albumin solution
0.5-1 g/kg over 4 hr
Furosemide 1-3 mg/kg/day
Add spironolactone 2-3 mg/kg/day
No response
(no weight loss or
diuresis in 48 hr)
Double dose of furosemide until diuresis,
or maximum daily dose
(4-6 mg/kg/day) is reached
No response
Add hydrochlorthiazide (1-2 mg/kg/day)
or metolazone (0.2-0.4 mg/kg/day)
No response
Furosemide IV bolus (1-3 mg/kg/dose)
or infusion (0.1-1 mg/kg/h)
No response
20-25% human albumin IV 0.5-1 g/kg
followed by IV furosemide 1-2 mg/kg
No response
Isolated ultrafiltration

Fig. 9 Management of edema in nephrotic syndrome. Patients with mild edema are managed with salt restriction; prednisone therapy is associated with spontaneous diuresis. Hypovolemia should be excluded (Table 4) before considering therapy with diuretics. Oral furosemide is the diuretic of choice; patients receiving therapy with furosemide for >48 h should additionally receive a potassium-sparing diuretic. Edema refractory to furosemide therapy may be treated with additional thiazide diuretics or IV furosemide, as bolus and/or infusion. Combination therapy with IV albumin (20%) and furosemide enables diuresis in patients refractory to the above measures. IV albumin carries the risk of fluid overload and pulmonary edema in patients with renal dysfunction. Patients with features of

Japanese patients, treated with immunosuppressive agents but currently normal cellular and humoral immunologic features, showed that immunization with live attenuated vaccines was immunogenic and safe [220]. Vaccination of family members is recommended. In cases where a patient has been in contact with a patient with varicella, prophylaxis with acyclovir or valaciclovir is advised [120, 221].

Thromboembolism

The risk of thromboembolism is variable, ranging from ~3% in children to ~20% in adults; children older than 12 years have a comparatively higher risk [222]. Venous thrombosis is more common than arterial thrombosis, and deep venous thrombosis, cerebral venous thrombosis, and pulmonary embolism account for the majority. The complication is chiefly observed in patients with frequent relapses, steroid dependence or steroid resistance. However, many patients show this complication early in the course of illness, more commonly during relapse than in remission [222]. Factors associated with this complication include increased platelet count, increased platelet aggregation, urinary loss of anticoagulant factors (anti-thrombin-III), and increased biosynthesis of coagulation factors (fibrinogen). The former two predispose to arterial, and the latter two to venous thrombosis. Prolonged bed rest, hypovolemia with hemoconcentration, infections, nephrotic range proteinuria, central venous catheters, and genetic variations favoring coagulation further increase the risk. Patients with marked hypoalbuminemia (<2 g/dL), low anti-thrombin-III (<70%), and high fibrinogen (>600 mg/dL) should be observed for the complication [215, 222]. If a thrombus is detected in a medium or large vessel, anticoagulation measures are instituted. Unfractionated or low-molecular-weight heparin is administered initially, followed by warfarin for 3–6 months. There is insufficient evidence for primary prophylaxis against venous thromboembolism in children with relapsing nephrotic syndrome.

Dyslipidemia

Nephrotic syndrome is associated with dyslipidemia. Hypoalbuminemia increases hepatic synthesis of lipoproteins, particularly low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) that increase total cholesterol, triglycerides, LDL cholesterol, and lipoproteins [223]. In SSNS, dyslipidemia rapidly normalizes during remission and is therefore unlikely to have long-term consequences, except for anecdotal reports on atherosclerosis and coronary artery disease. Since some patients might have additional cardiovascular risk factors (hypertension, obesity, insulin resistance), the potential risk of dyslipidemia cannot be ignored [216]. Use of lipid-lowering agents for dyslipidemia is not recommended, unless there is persistent proteinuria with extremely high levels of LDL cholesterol and triglycerides. Therapy with statins may be instituted in specific patients over 8-year-old, with monitoring of liver functions and creatinine kinase [223, 224].

Osteoporosis

Measurement of bone mineral density (BMD) using dual-energy X-ray absorptiometry is recommended for children with FRNS or SDNS [225, 226]. Bisphosphonates were reported to increase BMD during steroid therapy in children with nephrotic syndrome [227]. However, bisphosphonates may also reduce bone strength by inhibition of bone remodeling. A RCT in children with nephrotic syndrome, including patients with relapsing disease, suggested that administration of vitamin D and calcium supplements at initiation of corticosteroid therapy suppressed the decrease of BMD. However, the study also showed significant increase in levels of serum and urine calcium, leading to a risk for hypercalciuria and urolithiasis [228]. Collectively, there is insufficient evidence on bisphosphonates, vitamin D,

Fig. 9 (continued) hypovolemia require bolus(es) of normal saline if hypotensive, followed by oral and IV hydration, and IV albumin (20%) infused over 2–4 h. FENa fractional excretion of sodium; urine potassium index $UK^+/UK^+ + UNa^+$ (Modified from Indian Pediatrics 2021) and calcium for therapy of steroid-induced osteoporosis in children. The efficacy and safety of vitamin K, selective estrogen receptor modulators, and parathyroid hormone is not established.

Long-Term Outcome

About 25% patients experience only one episode of the disease and are cured after the initial course of corticosteroids. Fifteen to 20% patients experience relapses several months after stopping the treatment and a cure takes place after three or four episodes. The remaining 40–50% patients show either frequent relapses or steroid dependence. Patients with SDNS often have a prolonged course of their illness. However, if they continue to be steroid sensitive, the risk of progressive kidney disease is minimal.

There are few studies regarding the long-term outcome in of patients with SSNS starting during childhood. Studies from the 1980s reported that no more than 10% of children had further relapses in adulthood [229, 230]. More recent surveys indicate a relapse rate, after 18 years of age, to vary between 27% and 42% [231–233]. Risk factors for relapses during adulthood include a young age at onset, high number of relapses during childhood, and the need for use of alkylating agents and/or cyclosporine. Long-term therapy with calcineurin inhibitors may be detrimental to kidney function in adults.

The long-term side effects of treatments given to children have changed over the years. While corticosteroids and alkylating agents were the only drugs available for treatment of idiopathic nephrotic syndrome 30 years ago, there have been major changes in the approach for management of patients with FRNS with the availability of levamisole, mycophenolate mofetil, calcineurin inhibitors, and rituximab. Long-term steroid therapy started in childhood is associated with a number of adverse effects, mainly short stature, weight excess, osteoporosis, and cardiovascular events. Most recent studies on the final height showed an average height in adulthood close to the genetic target height and no detrimental effects of steroid therapy [233–235]. This may be due to the larger use of steroid-sparing agents when statural growth is impaired during childhood. The incidence of overweight in adulthood varies among the long-term cohorts, ranging between 19% and 35% [233]. However, these reports found no correlation between the body mass index and the cumulative dose of steroids or number of relapses. The incidence of overweight in these patients may be close to that observed in the general population.

Osteoporosis, associated with a risk of fractures, has been observed in significant proportion of adult patients, including patients who were still relapsing in adulthood [231, 233, 236, 237]. Long-term corticosteroid therapy may be associated with cataracts in ~10–33% children and 6–20% patients in long-term cohorts [234, 236–238]. A high incidence of systemic hypertension is found in adult patients receiving therapy with corticosteroids and/or calcineurin inhibitors [234]. The cardiovascular risks of long-term therapy with steroids, hypertension and dyslipidemia in patients with nephrotic syndrome are not known. Long-term toxicity of alkylating agents includes gonadal toxicity [239]. The risk of sterility is greater in boys than in girls. The cumulative threshold dose above which oligo/azoospermia may be feared is between 150 and 250 mg/kg [240].

SSNS is a self-limiting illness with the majority of patients outgrowing the disease by puberty. Review of information from multiple cohorts shows a considerable risk of disease persistence and long-term complications of the disease and/or its therapies [241]. Given these risks, it is advised to transfer care of adolescents with relapsing disease to “adult” nephrologists at the appropriate time. National and international guidelines advocate for smooth transition, with emphasis on shared clinics and consideration of patient and parent perspectives.

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Nongenetic Forms of Steroid-Resistant Nephrotic Syndrome

15

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Abstract

Historically, the classification of steroid-resistant nephrotic syndrome (SRNS) was based on histopathology alone, with minimal change disease and especially focal segmental glomerulosclerosis (FSGS) being the dominant lesions. Nowadays, genetic forms are differentiated from nongenetic forms. The latter can be broadly divided into two groups: (1) idiopathic SRNS is considered to be mediated by a (yet unidentified) permeability factor and (2) secondary forms of FSGS either resulting from a maladaptive response of podocytes to conditions promoting

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hyperfiltration (obesity, acquired solitary functional kidney, or uncontrolled hypertension) or distinct podocyte injury (e.g., viral infections, exposure to toxins or drugs). Histopathology is still an important diagnostic tool and can help to assess prognosis and recent studies have shown its potential as research tool in clarifying pathogenesis. Large studies and registry data have shown that overall prognosis of SRNS has improved in recent years because potent immunosuppressants are available. Especially the use of calcineurin inhibitors (in combination with steroids and RAAS inhibitors) is now recommended as first-line treatment option. For treatment of refractory patients, B-cell depleting antibodies (e.g., rituximab) and mycophenolic acid or pulse methylprednisolone are an option. Other interventions may be effective in individual patients. Unfortunately, patients unresponsive to treatment have a high risk of developing end-stage kidney failure and have a considerable risk of disease recurrence after kidney transplantation.

Keywords

Idiopathic SRNS · Secondary SRNS · Glomerular permeability factor · Pathophysiology · Histopathology · Immunosuppression · Calcineurin inhibitors · Recurrence · Columbia classification · Genetic SRNS · Rituximab · Mycophenolic acid · Methylprednisolone

Historically, the classification of the primary idiopathic nephrotic syndrome was based on histopathology alone [1]. Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) are the most frequent causes. Studies by the ISKDC, however, have shown that biopsies in patients responding to prednisone revealed MCD in more than 85% [2]. Thus, nowadays a biopsy is usually performed only in children with steroid-resistant nephrotic syndrome (SRNS) or when features suggesting another diagnosis are present (laboratory abnormalities, renal insufficiency, age >10 years, etc.). In recent years, more and more monogenetic forms of idiopathic SRNS have been described [3]. Approximately 80 different mutations are known and approximately 30% of SRNS patients are affected; some authors view genetic SRNS as a secondary cause [4, 5]. Response to immunosuppression, prognosis, and risk of recurrence after renal transplantation are different in genetic SRNS compared to those with acquired, i.e., nongenetic steroid-resistant nephrotic syndrome, although some overlap exists.

Pathophysiology

Studies from the early 1990s provide insights into the pathogenesis of FSGS. Once thought to be inactive cells, podocytes act as a buttress to the pressures within the glomerulus. Outward bulging of the glomerular basement membrane (GBM) initiates the well-described first committed step of focal glomerulosclerosis [6]. Podocyte injury leads to cell body attenuation, foot process effacement, pseudocyst formation, and microvillus transformation in the early phase of FSGS; interstitial fibrosis with tubular atrophy follows later [7, 8]. These common terminal lesions often provide little insight into the precedent injury. The identification of known podocyte-specific mutations has partially elucidated the mechanisms leading to the characteristic focal and segmental lesions in genetic forms of SRNS (see ▶ Chap. 10, "Genetic Basis of Nephrotic Syndrome").

Nongenetic forms of FSGS can be broadly divided into two kinds of disorders [9]. While idiopathic FSGS is considered to be mediated by an unidentified permeability factor, secondary forms of FSGS result from a maladaptive response of podocytes to conditions that either promote hyperfiltration, such as obesity, acquired solitary functional kidney, and uncontrolled hypertension, or lead to podocyte injury, such as viral infections (hepatitis B and C, parvovirus, HIV), exposure to toxins (e.g., heavy metals) or drugs (e.g., anti-VEGF monoclonal antibodies, lithium and interferon) [4].

Idiopathic FSGS

The term "idiopathic" FSGS describes a phenotype of SRNS in which FSGS lesions on the kidney biopsy are believed to be caused by a presumptive permeability factor, after other secondary factors have been excluded. Until the identification of such a permeability factor is universally accepted, the condition continues to be termed "idiopathic," harmonizing with global efforts to move away from the term "primary FSGS." Where the renal biopsy is associated with a FSGS lesion, with or without the presence of widespread podocyte foot process effacement, and a pathophysiologic mechanism is established (collectively known as "secondary FSGS"), treatment is often targeted at the underlying disease process. For this group, the term "secondary FSGS" will continue to be used, with the underlying cause explicitly articulated as far as possible, e.g., "secondary FSGS due to maladaptive responses" or "secondary FSGS due to other primary glomerular disease" [4].

Available information on the pathogenesis of idiopathic SRNS and FSGS nephrotic syndrome has led to various hypotheses, which are summarized in Fig. 1. A major breakthrough in understanding the pathogenesis of idiopathic SRNS was the identification of mutations in approximately 80 genes encoding structural elements of the slit diaphragm. The disease in the majority of the remaining cases is assumed to be mediated by immunological perturbations leading to generation of a circulating glomerular permeability factor. An imbalance between T helper 1 (Th1) and Th2 cytokines, increased Th17 versus T regulatory activity and enhanced urinary CD80 excretion by podocytes, chiefly reported in patients with steroid-sensitive nephrotic syndrome, require closer evaluation in SRNS [10]. A "circulating factor" hypothesis for SRNS is supported by observations that FSGS recurs in the allograft within hours of transplantation in 30–50% patients, resolves with plasmapheresis or following retransplantation in another individual, and by experimental data in which nephrotic plasma induces proteinuria in rats, increases permeability in isolated glomeruli, and induces cytoskeletal changes and/or cytoplasmic translocation of key slit diaphragm proteins [11, 12]. Investigations using various techniques indicate that the factor is absent from the lipid fraction, is denatured by heat, is destroyed by protease digestion, and weighs 30–50 kDa, indicating that it may be a low molecular weight protein. However, thus far, none of the reported circulating permeability factor candidates have been independently validated in primary FSGS [13]. Evidence favoring or disputing various candidate circulating factors have been published [11, 12, 14–16]. Within the span of a decade, soluble urokinase-type plasminogen activator receptor (suPAR), that had generated widespread excitement among researchers as a diagnostic biomarker representing the permeability factor in FSGS, has since been repurposed as a prognostic biomarker for chronic kidney disease (CKD) [17]. Cardiotrophin-like cytokine factor 1 (CLCF-1), a member of the IL-6 cytokine family that decreases nephrin expression in podocyte culture, was identified in the plasma of patients with FSGS, with concentrations in those with recurrent FSGS being up to 100 times that of normal subjects [11]. None of the current candidates have unequivocal evidence for role as the circulating factor. In addition, there could be different circulating factors in individual patients.

Maladaptive Injury Response

Several processes associated with intraglomerular hypertension (increased single-nephron GFR) are described to cause glomerular hypertrophy and podocyte activation, hypertrophy, stress, and denudation, leading to extracellular matrix deposition and glomerular sclerosis [18].

Obesity-Related Glomerulopathy

An association between obesity and nephrotic range proteinuria was first described in 1974 [19]. Various factors are implicated in the pathophysiology of obesity-induced glomerulopathy [20]. While increased renal plasma flow and hyperinsulinemia-induced efferent arteriolar

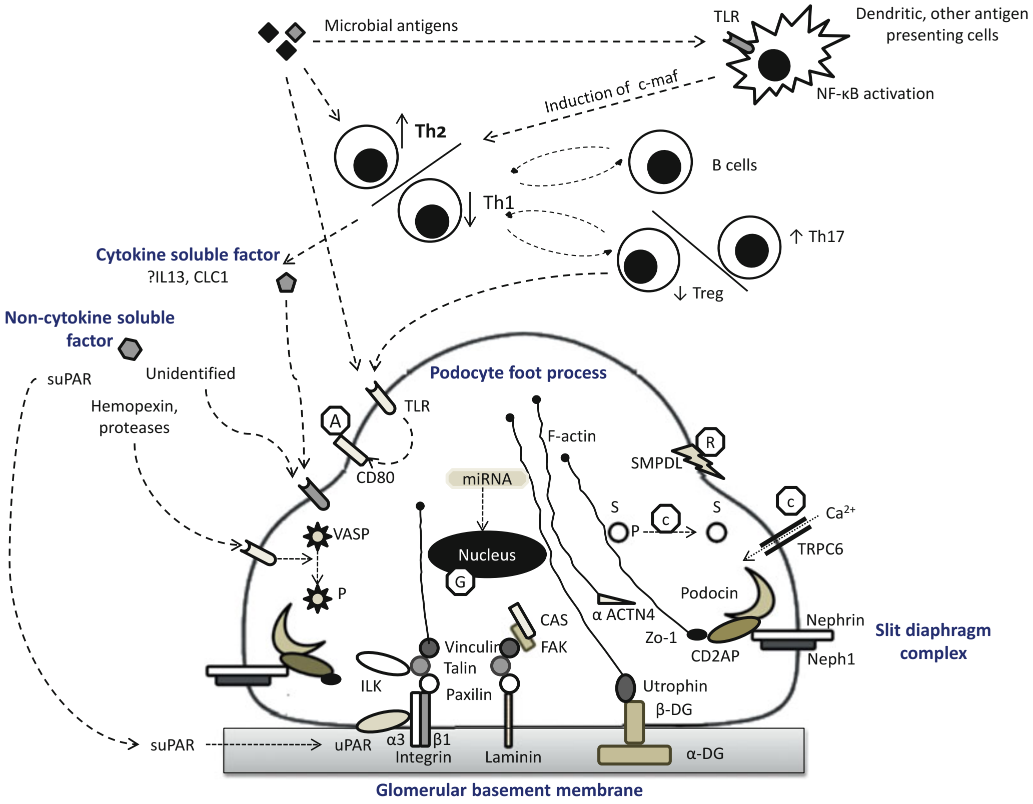
Microbial antigens
TLR
Dendritic, other antigen presenting cells
NF-κB activation
Induction of c-maf
↑ Th2
↓ Th1
B cells
↑ Th17
↓ Treg
Cytokine soluble factor
?IL13, CLC1
Non-cytokine soluble factor
suPAR
Unidentified
Hemopexin, proteases
Podocyte foot process
A
TLR
CD80
VASP
P
miRNA
Nucleus
G
F-actin
R
SMPDL
S
P
C
S
C
Ca2+
TRPC6
Podocin
α ACTN4
Zo-1
CD2AP
Nephrin
Neph1
Slit diaphragm complex
CAS
FAK
Vinculin
Talin
Paxilin
ILK
Utrophin
β-DG
suPAR
uPAR
α3
β1
Integrin
Laminin
α-DG
Glomerular basement membrane

Fig. 1 Prevailing hypothesis in the pathogenesis of idiopathic nephrotic syndrome of childhood. Schematic view of podocyte foot process demonstrates components of the slit diaphragm complex, formed by nephrin, Neph1, and podocin, and the actin cytoskeleton, which also receives inputs from the basolateral domain of the foot process, containing the a3b1 integrins and a and b dystroglycans. The dominant paradigm in nephrotic syndrome is an imbalance between T helper 1 (Th1) and T helper 2 (Th2) cytokines, and that a cytokine soluble mediator increases glomerular permeability. Non-cytokine molecules suggested as mediators include soluble urokinase plasminogen activator receptor (suPAR) that activates podocyte b3 integrin signaling to integrin-linked kinase (ILK), circulating proteases that phosphorylate vasodilator-stimulated phosphoprotein (VASP), vascular endothelial growth factor, and angiopoietin-like-4 (ANGPTL4). Signaling through nuclear factor kappa B (NF-jB) and toll-like receptor (TLR)-mediated pathways may polarize adaptive immune responses toward Th2 cells or directly increase CD80 expression in podocytes. An imbalance of Th17 and T regulatory responses might allow persistent CD80 activation on podocytes and/or helper responses. MicroRNAs may influence glomerular permeability through changes in gene transcription. Therapeutic agents are known to have direct effects on podocytes: glucocorticoids (G) on gene expression, calcineurin inhibitors (C) on stabilization of synaptopodin (S) or inhibition of TRPC6 channel, binding of rituximab (R) to the SMPDL-3b protein and of abatacept (A) to CD80. Modified with permission from Sinha A, Bagga A. Nephrotic syndrome. Indian J Pediatr 2012; 79: 1045–55

constriction lead to intraglomerular hypertension, hyperinsulinemia enhance the synthesis of growth factors like insulin-like growth factor 1, which promote glomerular hypertrophy. Elevated plasma leptin enhances transforming growth factor-β1 production that upregulates profibrotic pathways [21].

Low Nephron Mass

The impact of fetal programming on the development of adult disease has been of interest ever since Barker hypothesized that intrauterine growth restriction (IUGR), indicated by low birthweight, predisposes individuals to type 2 diabetes, hypertension, dyslipidemia, and cardiovascular disease in adult life. The strong epidemiologic relationship between intrauterine growth retardation and adulthood hypertension led Brenner to posit an inverse relationship between nephron mass and hypertension [22]. Birth weight is a strong determinant of nephron number and glomerular size [23, 24]. Data from infant autopsies suggests that newborns weighing less than the 10th percentile for the gestational age or below 2500 g have smaller kidneys, fewer nephrons, and larger glomerular volume than those with normal weight for age [86]. Since nephrogenesis in humans is completed around 36 weeks of gestation, prematurity also leads to low nephron number (oligonephronia) [25]. Overall, while IUGR appears to have greater impact on nephron mass than prematurity, both contribute to risk of proteinuric CKD. Several cohort studies have demonstrated disproportionate prevalence of albuminuria or biopsy-demonstrated FSGS in patients with low birth weight or IUGR [26].

Infection-Associated FSGS

Several viruses and parasites are known to be associated with FSGS. Proposed mechanisms include direct infection of podocytes and, rarely, activation of the innate immune pathways that lead to podocyte injury. Evidence of direct infection of the podocyte is demonstrated for HIV-associated nephropathy (HIVAN), where podocyte-restricted expression of HIV genes in transgenic mice was associated with collapsing glomerulosclerosis [27]. Chronic hepatitis B virus (HBV) infection may present with several different glomerular pathologies, ranging from primarily nephrotic conditions like minimal change disease (MCD), FSGS, and membranous nephropathy, to inflammatory conditions such as membranoproliferative glomerulonephritis and IgA nephropathy. Reduction in HBV viremia is associated with remission of proteinuria. Identification of viral DNA in urinary podocytes isolated from patients with collapsing variant of FSGS and chronic HBV infection indicates podocytes as a direct target for injury [28].

Parvovirus B19 DNA has been identified by in situ hybridization in kidney endothelial and visceral and parietal epithelial cells in various kidney lesions, including collapsing and idiopathic forms of FSGS, membranous nephropathy, and MCD [29] The histological picture mimics characteristic glomerular changes of HIVAN, including increased size and number of visceral epithelial cells, cytoplasmic vacuoles, and protein absorption droplets [30]. Parvovirus DNA was demonstrated in podocytes as well as tubular and parietal cells in cases with ultrastructure confirming morphologically altered podocytes [31], suggesting that, similar to HIVAN, parvovirus related collapsing FSGS reflects direct podocyte injury. In contrast, acute CMV infection, rarely associated with collapsing FSGS, is postulated to induce FSGS lesions through generation of a local interferon response, rather than direct viral cytopathic effect [32]. Acute EBV infection is also rarely described with FSGS; however, viral DNA has been consistently localized to kidney tubules rather than the glomerulus. Viral-associated kidney diseases are discussed elsewhere (▶ Chap. 27, “Viral Infections and the Kidney”). Recently, infections with COVID-19 have been associated with development of FSGS [33].

Toxic Exposures

Several case reports and case series highlight an association between nephrotic syndrome and exposure to heavy metals like mercury, copper, cadmium, and lead [34]. These associations are substantiated by in vitro and animal models demonstrating podocyte injury and proteinuria in response to exposure to heavy metals.

Drug-Induced Podocytopathy

Several medications have been associated with the development of FSGS. A collapsing variant of FSGS was first described following therapy with bisphosphonates in 2001 [35], with characteristic abundance of cells in Bowman space, which appeared to derive from parietal epithelial cells [36]. Monoclonal antibodies that inhibit the function of vascular endothelial growth factor (VEGF, e.g., bevacizumab, axitinib) induce proteinuria in a dose-dependent manner. VEGF is considered critical for glomerular endothelial cell and podocyte cross-talk, which is necessary to maintain normal podocyte structure. Hence, VEGF our leads to swelling and detachment of glomerular endothelial cells, vacuolization of endothelial cells, disruption of slit diaphragms, and downregulation of nephrin [35, 37]. Anthracyclines, like adriamycin and doxorubicin, are reported to lead to collapsing FSGS [38], and their injection in mice provides a reliable in vitro model for proteinuric disease [39, 40].

Therapy with interferon α, β, and γ is associated with minimal change, FSGS, or collapsing FSGS [41, 42]. While the precise mechanism is unclear, interferons may affect podocyte proliferation, cellular metabolism or oxidative capacity, and/or increase the expression of major histocompatibility complex (MHC) class II antigens that increase production of interleukin (IL)-6 or −13, leading to macrophage activation [43]. The long-term use of anabolic steroids was associated with FSGS in nine of ten bodybuilders, along with glomerulomegaly in four cases. Podocyte injury was considered secondary to post-adaptive glomerular changes driven by increased lean body mass and/or direct nephrotoxicity of anabolic steroids [44]. Heroin-associated nephropathy, reported in 1970s–1990s in IV drug abusers, was characterized histologically by either FSGS or amyloidosis; however, causality was not confirmed since contaminants (e.g., scopolamine), concomitant viral infections (e.g., HCV or HIV), and most recently, *APOL1* genetic variants are also implicated [45]. Chronic lithium toxicity, which typically presents with nephrogenic diabetes insipidus, and is histologically characterized by chronic tubulointerstitial nephropathy and distal tubular cysts, may uncommonly be associated with minimal change disease or FSGS [46, 47]. Pathogenesis might involve lymphocyte-derived factors that alter the filtration barrier or interactions of lithium cations with the negatively charged GBM [48]. While some cases with FSGS have glomerulomegaly, suggesting secondary FSGS, others have features consistent with a primary podocytopathy. Other drugs associated with nephrotic range proteinuria are nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors (minimal change disease) and sirolimus (FSGS) [49].

Pathology

Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) are the most frequent causes of nephrotic syndrome in children. Patients with MCD usually respond to glucocorticoids, while FSGS accounts for approximately 90% of SRNS; Therefore, FSGS and SRNS are often referred to synonymously. It should be remembered, however, that patients with SRNS may have MCD, although some authors regard this as yet undiagnosed FSGS. Lastly, some patients respond to corticosteroids initially but then develop secondary steroid-resistance and may have MCD or FSGS on kidney biopsy.

In FSGS, other pathophysiological factors are causative and multiple etiologies have been described above. However, in the majority of nongenetic SRNS cases, the underlying mechanism is unknown [9]. Therefore, a histopathological diagnosis of FSGS (and MCN) has to be viewed as a lesion or histological pattern and not as a disease itself as findings are nonspecific. Although some histopathological variants correlate with certain etiologies, no systematic studies have been performed in order to address differences among these, e.g., between genetic and nongenetic (especially immunological causes). Patients with ACTN4 mutations demonstrate distinctive biopsy features suggestive of maladaptive FSGS [9]. Nevertheless, renal biopsy is still an important tool in the evaluation of a child with SRNS and specific clues can be provided by light microscopy, immunofluorescence, and electron microscopy.

Histopathology (Fig. 2)

Because most patients with nongenetic SRNS have MCD or FSGS, focus will be on these two entities. In MCD, **light microscopy** will reveal normal-appearing glomeruli with normal cellularity and capillary walls. Mild focal mesangial predominance may be seen; if more than 3 mesangial cells/per mesangial area away from the vascular pole region are present in at least 80% of

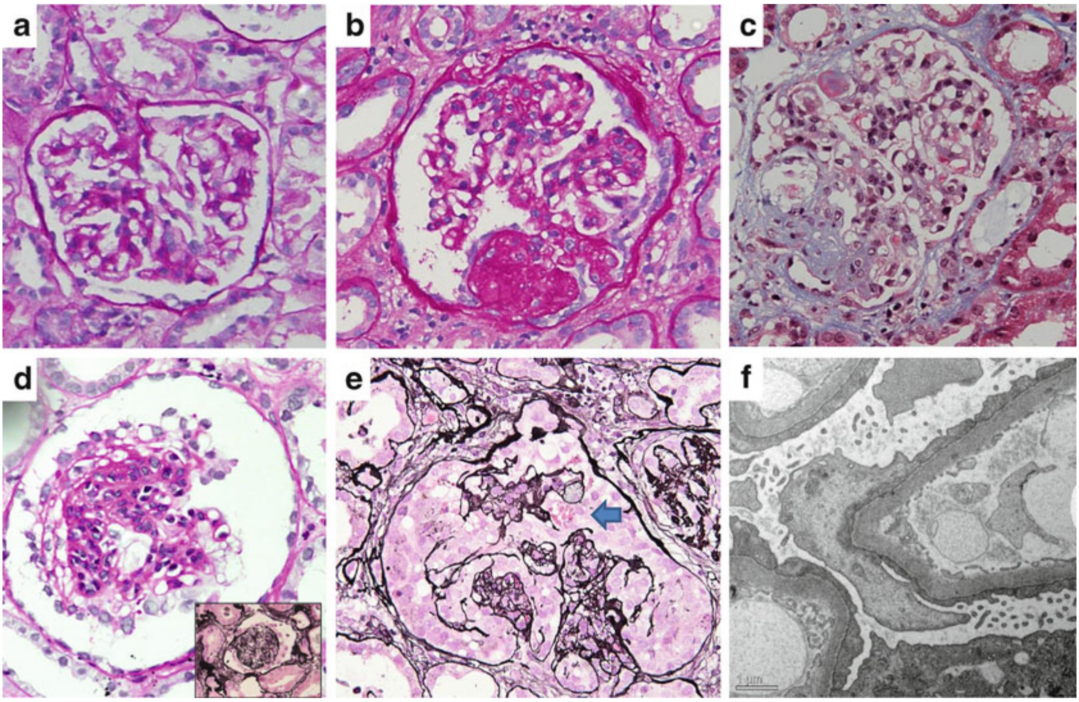

Fig. 2 (**a**) Minimal change: Morphological unremarkable glomerulus (periodic acid-Schiff; 20×). (**b**) Focal segmental glomerulosclerosis not otherwise specified (FSGS-NOS): The sclerotic segment at 6 o'clock is "crowned" by epithelial cells. Note the irregularly thickened Bowman capsule (periodic acid-Schiff; 20×). (**c**) FSGS-NOS: The collagenized sclerotic segment at 8 o'clock stains blue, while peripheral hyalinosis noted at 11 o'clock position stains pink (Masson trichrome; 20×). (**d**) Diffuse mesangial sclerosis: The mesangium appears sclerotic and hypercellular without open capillary loops and with variable overlying visceral epithelial cell proliferation (periodic acid-Schiff; 20×). Inset shows classic mesangial knotting or "yarn ball" appearance and crowning with a single layer of visceral epithelial cells (Jones methenamine silver; 20×) (**e**) Collapsing glomerulopathy: The glomerular tuft is globally collapsed or imploded by markedly hypertrophic and hyperplastic visceral epithelial cells, displaying conspicuous nucleoli and protein resorption droplets (arrow) (Jones methenamine silver; 20×) (**f**) Transmission electron microscopy, in all primary podocytopathies, is characterized by diffuse foot process effacement, actin condensation, and microvillous transformation, the ultrastructural hallmarks of podocyte injury (uranyl acetate-lead citrate; 2550×). (Images courtesy Dr. Geetika Singh, Department of Pathology, All India Institute of Medical Sciences, New Delhi, India)

glomeruli, this is termed diffuse mesangial hypercellularity variant of MCD.

In FSGS of the usual, not otherwise specified type (nos), a segmental increase in the glomerular matrix with obliteration of the capillary lumina is seen [50]. The first characteristic is that glomerular lesions in FSGS affect only some of the glomeruli in early stages of the disease. Of note, in advanced FSGS, the lesions may rather involve all glomeruli. Usually juxtamedullary/inner cortex glomeruli are affected first. Detailed serial section studies show that even though the extent of segmental lesions increases with such three-dimensional analysis, the lesions remain focal, i.e., sparing some of the glomeruli, even in patients with moderately advanced disease [51]. As focality is more pronounced in children, comprehensive sampling is critical especially in this age group. As a second characteristic, the sclerosis is segmental, affecting only a part of the glomerular tuft. In early stages, capsular adhesions without underlying abnormalities in the tuft may be present and indicate a diagnosis of early FSGS rather than MCD. Nonsclerosed glomeruli show significant glomerular hypertrophy in FSGS, compared to age-matched controls. In some instances, the whole tuft is affected by sclerosis (focal and global sclerosis). Global glomerulosclerosis in an obsolescent pattern, i.e., the tuft condensed and globally sclerosed with a corona of matrix filling in Bowman's space, is a nonspecific lesion, present in normal individuals and increasing with age. Even young children may thus show occasional global glomerulosclerosis in this pattern, and this does not per se indicate a diagnosis of FSGS.

Sclerosis may progress and plasma insudation may be present, evident by glassy, smooth eosinophilic and PAS-positive material (hyalinosis). This lesion is nonspecific and may be seen in any sclerosing injury. There may be vacuolization of podocytes. Detachment of podocytes from the basement membrane is typical in children, best viewed by electron microscopy. Mesangial hypercellularity may be present in children with FSGS, but is not per se sufficient to diagnose FSGS rather than MCD (see above) if segmental sclerosing lesions or adhesions are not present. Tubular changes are often present and may show patchy atrophy, interstitial fibrosis, and lymphocytic infiltration correlating with glomerular pathology. Tubulointerstitial fibrosis in the absence of segmental sclerosing lesions raises suspicion for unsampled FSGS. Additional serial sectioning may then be undertaken to hunt for diagnostic glomerular lesions. Lipids and protein droplets may be present in proximal tubular cells.

Immunofluorescence

Immunofluorescence is usually negative, although mild staining for C3, C1q, IgM, or IgA may occur in mesangial areas in MCD, particularly if mesangial hypercellularity is present, and in sclerosed areas with or without hyalinosis in FSGS. The presence of more than trace amounts of IgG and IgA is suggestive of an immune complex process rather than just podocyte injury, especially if cellular proliferation is also present. The presence of IgM staining by IF but without deposits by electron microscopy was associated in some early studies with poor response to corticosteroids and some authors suggested the term IgM nephropathy. More recent work suggested that IgM deposition is more frequent in FSGS than in MCD but it has also been described in IgA nephropathy and in areas of mesangial increase in MCD, thus being nonspecific. Deposition of IgM, which is a large molecule, may be due to trapping in an injured glomerulus; others have suggested that IgM binds to neoepitopes in the mesangium after nonimmune injury resulting in complement activation [52]. Therefore, IgM nephropathy remains a controversial entities and more work has to be done to define the distinct pathogenesis and clinical implications of these lesions.

In some biopsies of NS patients, staining for C1q is dominant greater than other components, with mesangial and occasional subendothelial deposits on electron microscopy. This constellation of findings has been termed C1q nephropathy. Systemic lupus erythematosus and other disorders of complement activation need to be excluded, as these may also lead to C1q deposits. This lesion is of unknown etiology and shows variability both histologically as well as clinically, including therapeutic responses and prognosis. The light microscopic pattern may show no lesions, or proliferative lesions, or segmental sclerosis, and has been correlated with clinical course. Patients with minimal lesions by light microscopy have done well, whereas those with segmental sclerosing lesions have followed a course similar to FSGS, and those with proliferative lesions behaved more like immune complex disease. In a series of 12 pediatric patients with C1q nephropathy, light microscopy showed FSGS with or without diffuse mesangial proliferation in 6, MCD in 4, and focal proliferative glomerulonephritis in 2 patients. While C1q deposits were found in all patients, electron microscopy revealed visible deposits only in nine cases. The response to steroids was variable and steroid resistance was associated with poor prognosis [53].

The involvement of the complement system in FSGS is also supported by a recent study demonstrating Cd4 positive staining in 73% of FSGS patients compared to 21% of MCN and 10% of controls [54]. C4d is generated by the activation of the classical and lectin pathways, and hence its presence can point to the activation of either of these pathways. Interestingly, fibrosis was absent in 42% of patients with C4d+ staining and was more prevalent in patients who developed recurrent FSGS after transplantation. In rats, CD4 staining was frequently positive at the onset of proteinuria and before the FSGS lesions developed, suggesting a role of complement activation in the early stages of FSGS. In another study, mesangial C4d deposition was an independent predictor of disease progression [55]. More studies are warranted to define the role of the complement system in FSGS.

Electron microscopy

Electron microscopy plays an important role in the investigation of patients with SRNS since ultrastructural changes at the podocyte level are present irrespective of the etiology or changes on light microscopy. EM also is also an important tool to exclude immune complex disorders and metabolic causes such as amyloidosis or Fabry disease. Some genetic forms of SRNS such as COL4 mutations are associated with distinct basement membrane alterations, i.e., thinning of the glomerular basement membrane early, with later segmental thickening and basket-weaving (see chapter on Alport/thin basement membrane lesion) which are assessed by EM. Foot process effacement (FPE) is extensive in MCD and so-called primary FSGS, even in nonsclerosed glomeruli; this may be the only morphological lesion in MCD and correlates with the degree of proteinuria. Of note, occasionally a biopsy is planned for a patient with relapsing NS, and corticosteroid treatment is initiated before biopsy. If the patient has a partial response by the time of biopsy with decreased proteinuria, there may be limited or no FPE on biopsy, with no segmental lesions by LM. In this clinical context, with a patient who had NS some days or weeks early, who has been treated, this constellation of clinicopathologic findings is consistent with treated/responsive MCD.

Global FPE and foot process width are overall greater in primary idiopathic FSGS compared to maladaptive FSGS. However, extensive FPE may be seen in secondary FSGS, particularly if the glomerulus assessed by EM at some level has segmental sclerosis. Thus, FPE less than around 50% is not consistent with untreated primary FSGS, and extensive FPE may represent either primary or secondary forms of FSGS. Sclerotic lesions show a wrinkled basement membrane, obliteration of the capillary lumen and may show hyaline (see above), seen by EM as paramesangial and subendothelial, finely granular, osmiophilic densities. The presence of lipid droplets, their location in sclerotic areas, and correlation with the EM scout section will avoid the mistake of confusing these with immune complex deposits. Foamy macrophages in sclerosed areas may contain protein and lipid droplets.

The Columbia Classification of FSGS and Clinical Relevance

For clinicians, the development of the Columbia classification of FSGS has helped to translate complex histopathological findings into clinical practice [56]. It shows good reproducibility among independent observers and includes five variants:

1. Most frequent is the classical or “not other specified” (nos) FSGS.
2. FSGS with lesions at the vascular pole of the glomerular tuft (perihilar type).
3. The cellular variant (podocyte hyperplasia, endocapillary hypercellularity, foam cells, and leucocyte infiltration).
4. Lesions at the tubular pole (tip lesion).
5. The collapsing variant.

It should be noted that the segmental lesions may show mixed appearances in a given biopsy, and the Columbia classification thus proposed a hierarchical classification schema. Further, with progressive scarring, all patterns may develop into progressive scars of nos type, or global sclerosis. Further, none of the patterns are unique for so-called primary FSGS, and insights have emerged suggesting specific etiologies linked to specific morphologic class. Several retrospective studies have correlated clinical outcome with the Columbia histopathological class. Overall, the tip lesion often has a favorable outcome, similar to MCD, with response to therapy, contrasting the much worse outcome seen with the collapsing variant. The collapsing variant is often associated with infections, such as HIV, parvovirus B19, CMV, and now even recognized to occur with COVID-19 [33]. Drugs may also cause this lesion, including, e.g., bisphosphonates, interferon, anabolic steroids, TNF alpha antagonists, and others. Idiopathic collapsing glomerulopathy predominates in patients of Black African ethnicity especially those with APOL1 risk allele variants (G1, G2, compound heterozygous or homozygous) and is associated with a poor prognosis. Recurrent FSGS in the transplant generally, but not always, shows fidelity to the pattern of the original native kidney lesion [57].

Of interest, in the NIH multicenter trial of steroid-resistant FSGS patients, 138 patients age 2–38 years were randomized to cyclosporine or mycophenolate mofetil with dexamethasone [58]. Classical (nos) FSGS was the most frequent lesion (68%) followed by collapsing (12%), tip (10%), perihilar (7%), and the cellular type (3%). Proteinuria in (nos) FSGS was more likely to be subnephrotic. Tip or collapsing lesions were more frequent in teenagers and adolescents and subjects with these variants had greater proteinuria and hypoalbuminemia than those with classical FSGS. The tip variant was more common in whites and was associated with lower injury scores and rate of progression. In contrast, the collapsing variant, which was more frequent in African Americans, carried an unfavorable prognosis. The rate of progression to end-stage kidney disease was highest in collapsing (47%), intermediate in NOS (20%), and lowest in the tip variant (7%). This is notable, as these tip patients were nonresponsive to steroids, and thus not representative of typical tip lesion patients who respond to immunosuppression. Unfortunately, this study did not include tests to diagnose genetic causes or APOL1 polymorphisms.

A recent study evaluated the prognosis of collapsing versus classical (nos) FSGS controlling for treatment [59]. Of 187 patients, 61 had collapsing and 126 not otherwise specified lesions. Despite having lower eGFRs and higher levels of proteinuria, patients with collapsing FSGS had similar outcomes in terms of patients achieving remission or those progressing to end-stage renal disease. Although patients with collapsing FSGS had higher levels of proteinuria and a lower eGFR, the outcome was similar between groups, regarding proportion of patients reaching remission or end-stage renal disease. Thus, controlling for severity of disease at presentation, collapsing was not worse – the challenge being that collapsing patients in multiple other series had worse features at presentation. The challenge is to identify and diagnose such patients at earlier time points. The positive impact of early treatment has also been demonstrated in recurrent FSGS after transplantation, where new treatment options initiated at very early time of recurrence have improved prognosis.

Systematic evaluation of certain histopathological findings is difficult to perform or at least time-consuming. This, for instance is especially true for distinct features in FSGS, e.g., glomerular hypertrophy and tubular fibrosis. Both have been suggested as markers of an unfavorable prognosis, but no large studies are available that evaluated the prognostic relevance in details. This problem could be resolved in the near future with the help of deep learning. Renal pathology is just entering the era of digital pathology and computational image analysis, allowing for tissue-interrogation not previously possible.

Deep learning-based segmentation of histological structures has been performed. In a recent study by Jayapandian et al. [60], 125 renal biopsies from MCNS patients collected in the Neptune database were analyzed. They demonstrated that periodic acid-Schiff staining yielded best concordance between pathologist and deep learning segmentation across all structures (glomerular tuft, proximal tubules, distal tubular segments, peritubular capillaries, and arteries and arterioles). Although machine learning will never replace the experience of a histopathologists, large-scale evaluation especially of quantitative changes of specific structures will be possible in adults and children with (genetic and nongenetic) FSGS and may help to identify prognostic factors [61].

Research Directions of Histopathology

MCD and FSGS have historically been viewed as one entity, as progression from apparent MCD to FSGS occurs in some patients; also in some patients with FSGS, treatment can induce sustained remission, even after discontinuation, suggesting that FSGS can regress in MCD [62]. It is also known that there is complex interaction between resident cells of the glomerulus leading to FSGS (Table 1).

However, detailed immunostaining, morphometry, and even proteomic studies reveal differences in the biopsies of those patients without diagnostic segmental sclerosing lesions even in this presclerotic phase of FSGS versus MCD. These changes include increased expression of markers of activated parietal epithelial cells on the glomerular tuft (e.g., CD44) and marked glomerular hypertrophy. When FSGS recurs in the transplant, evidenced by marked proteinuria developing as early as in the operating room, the earliest structural lesion is only foot process effacement. However, additional studies reveal increased CD44 staining on the tuft, followed by overt sclerosing lesions within weeks [63]. Thus, although foot process effacement is extensive in both MCD and the early presclerotic phase of FSGS, there are important differences even at these earliest stages of podocyte injury. Whether this represents differences in severity of the same injury, or at least in part differing injury mechanisms, remains controversial.

Other changes of parietal epithelial cell phenotypes – even in unaffected glomeruli – have been demonstrated. In the collapsing variant, normal-appearing glomeruli had more claudin and CD44 positive cells and less WT1 in tuft area compared to controls. In contrast, more WT1 was found in tip lesions. A recent study demonstrated that glomerular epithelial lesions can be formed not only from the classical flat but also from proximal tubular epithelial cells: intermediate (located at the tubular orifice) and cuboidal PECs [64]. In mice, these cells expressed activation markers (CD44 and KI-67) and formed sclerotic lesions. Patients with FSGS and tip lesions expressed markers of intermediate PEC, which can be explained by the localization of these cells at the tubular pole. In summary, epithelial cells may not only contribute to the formation of sclerotic lesion but also to the colonization of unaffected glomeruli thus promoting lesion formation. Staining for parietal epithelial markers, such as PAX8, may also become a tool in diagnostic histopathology [65].

The formation of extracellular matrix is the final event. Increased numbers of pro-fibrotic molecules such as angiotensin, its pro-fibrotic AT1 receptor, TGF-ß, and various others have been demonstrated in FSGS. A recent study used proteomics to study the extracellular matrix composition in human biopsies in collapsing and classical FSGS [66]. Extracellular matrix proteins were more abundant in the collapsing than in the classical variant, especially transcripts of cathepsin B, C, and annexin. Interestingly, expression markers of epithelial cells but not of podocytes were demonstrated, again underlining the important role of parietal cells, also as a possible therapeutic target.

Taken together, histopathology not only plays a major role in the diagnosis (or exclusion) of

Table 1 Resident cells of the glomerulus and FSGS

Resident cell type	Role in pathogenesis
Glomerular endothelial cell	Endothelial cell-podocyte cross talk: Glomerular endothelial mitochondrial oxidative stress and dysfunction leads to podocyte loss
Parietal epithelial cell	Activation leading to hypertrophy, hyperplasia, and migration from Bowman space to the glomerular tuft, with subsequent increased production of extracellular matrix
Visceral epithelial cell (podocyte)	Disruption of cell cytoskeleton causes effacement leading to loss of integrity of foot processes and slit diaphragm
Mesangial cell	Injury and hypercellularity often found but unclear if primary or secondary mechanism

FSGS but also is a strong instrument to clarify distinct pathogenetic mechanisms of podocyte dysregulation leading to extracellular matrix formation; ultimately, this will also open the window for more effective treatment.

Management

The management of patients with SRNS is challenging because of variable response to immunosuppression, therapy- and disease-related adverse effects, risk of progression to chronic kidney failure, and likelihood of posttransplant recurrence. With any immunosuppressive strategy, the intent is to induce complete remission of proteinuria (protein/creatinine ration <0.2 mg/mg), which is associated with favorable long-term outcomes [67]. However, also achievement of a partial remission has been shown to improve prognosis [68]. This is often defined as proteinuria of >0.2–2 mg/mg creatinine or cessation of edema and increase of serum albumin >25 g/l. A suggested definition of nonresponse is persistence of proteinuria >2.0 or presence of edema with serum-albumin <25 g/l [69].

Based on limited information from clinical trials examining the efficacy of immunosuppression in inducing and maintaining remission, the current standard of care for childhood SRNS comprises of a combination of treatment approaches, including calcineurin inhibitors, alternate day prednisone, and adjunctive measures such as RAAS blockade [70]. It should be emphasized, however, that in the past, no prospective study has differentiated between idiopathic steroid-resistant nephrotic syndrome and genetic forms. Patients with genetic forms (including those with APOL1 risk variants) have an inferior response to immunosuppression and testing has not been performed even in some recent controlled studies. Therefore, previous studies should be interpreted with some caution. In addition, publication bias and geographical differences reflecting differences in genetic background have to be taken into account, since they might have an impact on treatment response. A suggested management algorithm is presented in Fig. 3.

Immunosuppressive Regimens

Pulse Corticosteroids

In view of the poor prognosis in the pre-CNI era, early studies had examined the utility of IV methylprednisolone (20–30 mg/kg for 6 alternate days; then weekly for 8 doses, fortnightly for 4 doses, monthly for 8 doses, and bimonthly for 4 doses) or IV dexamethasone (4–5 mg/kg for 6 alternate days, then fortnightly for 4 doses, and monthly for 8 doses), usually along with oral cyclophosphamide [71, 72]. While complete or partial remission were observed in 30–50% cases, high risk of steroid toxicity (hypertension, hypokalemia, hyperglycemia, steroid psychosis) and systemic infections are reasons why these are no longer recommended in management of steroid resistance [73]. Recently, however, combination of a calcineurin inhibitor with pulse methylprednisolone was able to achieve sustained remission in 84% of children in a single center study so that this approach might be an alternative for individual refractory patients [74].

Calcineurin Inhibitors (CNI)

Calcineurin inhibitors suppresses IL2-mediated T-cell activation and have been used in the treatment of nephrotic syndrome since the mid-1980s. In addition to its immunosuppressive action, cyclosporine has been found to have a direct hemodynamic effect and is able to stabilize the actin-cytoskeleton of podocytes; these two effects may be the explanation for its effect in non-immunologic, genetic SRNS [75].

Calcineurin inhibitors along with alternate day steroids are currently viewed as the first-line treatment of SRNS. Information from 9 RCTs examining the efficacy of cyclosporine or tacrolimus for SRNS indicates that these agents induce remission in up to 70% cases (250 patients; 95% CI 64.1–75.3%), which is more than reported for other therapies. Meta-analysis indicates that use of either CNI is associated with higher likelihood of remission at 6–12 months versus no treatment (2 RCT; RR 3.5; 95% CI 1.0–9.6) or therapy with IV cyclophosphamide (3 RCT; RR 1.98; 95% CI 1.25–3.13) [5]. Cyclosporine and tacrolimus have similar efficacy (2 RCT; RR 1.1; 95% CI 0.9–1.3)

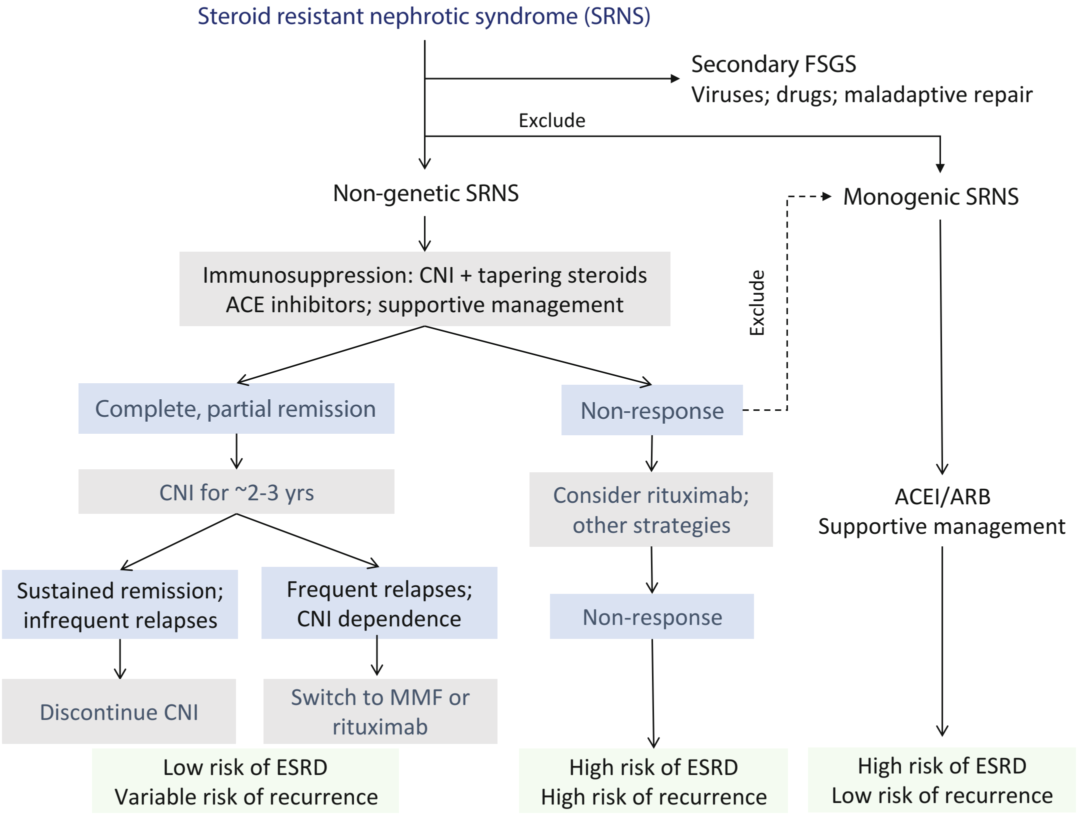
Steroid resistant nephrotic syndrome (SRNS)
Secondary FSGS
Viruses; drugs; maladaptive repair
Exclude
Non-genetic SRNS
Monogenic SRNS
Immunosuppression: CNI + tapering steroids
ACE inhibitors; supportive management
Exclude
Complete, partial remission
Non-response
CNI for ~2-3 yrs
Consider rituximab; other strategies
ACEI/ARB
Supportive management
Sustained remission; infrequent relapses
Frequent relapses; CNI dependence
Non-response
Discontinue CNI
Switch to MMF or rituximab
Low risk of ESRD
Variable risk of recurrence
High risk of ESRD
High risk of recurrence
High risk of ESRD
Low risk of recurrence

Fig. 3 Outline of principles of management in pediatric patients with idiopathic steroid-resistant nephrotic syndrome (SRNS)

and comparable risk of nephrotoxicity or hypertension [70]. Data collated in disease registries report lower rates of remission (41–76% patients), possibly due to selection bias, attrition, and confounding [67, 76].

Both the IPNA and KDIGO guidelines recommend first-line use of CNI for patients with SRNS [6, 8, 77]. Cyclosporine and tacrolimus are available options and seem to be equally effective [78]. Selection of either treatment partly depends on local preference and experience as well as consideration of side effects. While tacrolimus lacks the cosmetic adverse effects, cyclosporine may be preferred in patients with seizures or at-risk of diabetes. Dose titration is essential with either agent since bioavailability is variable and therapeutic index narrow; under- and overdosing might lead to nonresponse and toxicity, respectively. Therefore, close pharmacokinetic monitoring should be employed, typically aiming at trough levels of 80–150 ng/mL for cyclosporine and 4–8 ng/mL for tacrolimus.

The median time to remission is 2–4 months [70]; patients with nonresponse despite therapy with CNI in adequate doses for 6 months can be considered for other immunosuppressive strategies including discontinuation of CNI [73], unless they have achieved partial remission. It should be noted, however, that individual patients achieve complete remission only after years [62]. Patients in sustained remission for more than 6 months may be managed on lower doses [79]. Therapy should be withheld during episodes of acute kidney injury (AKI) or in patients with persistently low eGFR.

The optimal duration of treatment with CNI for patients who achieve partial or complete remission is not determined. In view of the poor prognosis of SRNS, evidence-based guidelines usually recommend therapy for 1 year or longer [73, 80]. Most patients would be continued on a CNI for 2–3 years or even longer before attempting withdrawal. In one study, discontinuing CNI has been shown to be feasible in the majority of patients in sustained remission [62], but may risk relapse. Other weaning protocols have been used, e.g., with MMF [81] or rituximab [82]; however, no randomized controlled studies are available. On the other hand, long-term CNI therapy beyond carries a risk of irreversible chronic nephrotoxicity, characterized by striped pattern of tubule-interstitial fibrosis and peripheral arteriolar nodular hyalinosis [11]. Risk factors for histological nephrotoxicity, observed in 10–25% cases treated for 2 years or longer, include high CNI dose, persistent heavy proteinuria, and hypertension [83, 84].

Patients who attain remission with CNI but continue to experience disease relapses despite adequate trough levels are considered for switch to alternative therapies. Retrospective studies indicate successful switch from therapy with cyclosporine or tacrolimus to mycophenolic acid, usually mycophenolate mofetil (MMF) or rituximab (discussed below). However, a recent randomized controlled trial found continued therapy with tacrolimus twice as effective in maintaining remission compared to switch to MMF after 6 months (90% vs. 45%) [85].

Mycophenolate Mofetil (MMF)

MMF is the prodrug of mycophenolic acid (MPA), an inosine monophosphate dehydrogenase inhibitor that interferes with de novo purine biosynthesis in proliferating B and T-lymphocytes. It also inhibits lymphocyte recruitment, myofibroblast infiltration, mesangial proliferation, and podocyte apoptosis. Given its lack of renal, hemodynamic, and metabolic adverse effects, MMF was tested as a safe alternative to CNI. However, contrary to the efficacy of MMF in patients with frequently relapsing steroid-sensitive disease, response is less satisfactory in patients with SRNS [86].

MMF was used in pediatric patients with SRNS in 9 prospective and retrospective case series, as summarized recently. Therapy with MMF induced complete or partial remission in 56 (62.2%) of 90 patients included in 4 studies. In 5 additional studies including 35 patients with CNI-dependent disease, MMF sustained remission when CNI was discontinued [87]. However, remission was sustained in only 17% cases treated with MMF in the PODONET cohort [67]. In the NIH-funded multicenter open label FSGS Clinical Trial, complete or partial remission was observed in 22 (33.3%) of 66 patients receiving MMF oral pulse dexamethasone, as compared to 33 (45.8%) of 72 randomized to receive cyclosporine [88]. A single-center RCT found lower remission rate in patients switched to MMF after 6 months of therapy with tacrolimus, compared to those continued on tacrolimus (44.8% vs. 90.3%) [85].

One lacuna in past studies was the lack of attention to therapeutic drug monitoring. An area under the curve >45–60 μg*h/mL is shown to be an important determinant of response to MMF in patients with steroid-sensitive nephrotic syndrome [89]. Future studies examining the efficacy of MMF in inducing remission of SRNS or in maintaining CNI-induced remission of proteinuria should ensure targeting adequate MPA levels. Therapy with MMF is useful in maintaining remission in patients with CNI-dependence and/or features of toxicity. Close monitoring for adverse effects, including gastrointestinal disturbances and myelosuppression, is necessary, particularly at high doses (▶ Table 3, Chap. 14, “Steroid-Sensitive Nephrotic Syndrome”) [90].

Rituximab

In the last two decades, rituximab has emerged as an effective strategy for sustaining remission in patients with steroid- and calcineurin inhibitor (CNI)-dependent nephrotic syndrome [69, 91]. Binding of this monoclonal antibody to CD20 on the B lymphocyte surface induces apoptosis and B-cell depletion, which might alter T-cell function and restore the balance between T-regulatory and Th17 cells [92]. The agent is also postulated to act directly on podocytes by upregulating sphingomyelin phosphodiesterase activity to stabilize the actin cytoskeleton [93].

In their first report in 2007, Bagga et al. reported an excellent response to rituximab in all five treated patients, including three with FSGS. Three patients achieved complete remission, and two partial remission [94]. Further studies indicated, however, that rituximab appears to have limited efficacy in inducing remission in patients with CNI-refractory SRNS as compared to steroid-sensitive nephrotic syndrome [95]; no response was seen in 70.7% of patients with SRNS, and there was a higher proportion with MCD that showed remission compared with FSGS [96]. A randomized controlled trial on 31 children with CNI-refractory SRNS showed no reduction of proteinuria at 3 months and 6 months following addition of rituximab to therapy with steroids and CNI. However, a remission of proteinuria was noted in six patients with delayed resistance (i.e., patients with secondary steroid resistance), indicating that this subgroup may respond differently [97].

In a multicenter open label single-arm trial, 9 (39.1%) of 23 patients with CNI-refractory SRNS achieved remission within 6 months of treatment with rituximab [98]. A review of therapy with rituximab in 234 pediatric patients with refractory SRNS (13 case reports, 10 case series) showed remission in 50.4% (range 18.8–80%) patients, including complete remission in 29.8% and partial remission in 22.9% cases [99]. A systematic review, including 226 patients with CNI-refractory SRNS (7 case series and 1 RCT), reported remission in 46.4% cases [100]. Further, these reviews indicated a trend to higher rates of response to rituximab for patients with minimal change versus FSGS, and for those with late versus initial resistance; patients with partial remission also seemed to have a better response to rituximab in a retrospective analysis Mason [101]. However, remission rates in uncontrolled studies might have been overestimated due to the inclusion of an admixture of CNI-dependent and CNI-resistant patients, effect of concomitant immunosuppression, and short follow-up.

Despite limitations, many pediatric nephrology centers use intravenous rituximab in selected patients of steroid- and calcineurin inhibitor-resistant nephrotic syndrome. The dose and frequency of administration is not defined. A range of doses from 375 mg/m^2 (single, or up to four doses) to 750 mg/m^2 per dose (two doses given 7–14 days apart) have been used. No head-to-head studies comparing different dosing schedules have been performed in SRNS (in contrast, e.g., compared to steroid-sensitive nephrotic syndrome, where a difference between intensive and moderate dosing schedules did not show a difference in response rates.) The pharmacokinetics of rituximab are altered in proteinuric patients; enhanced drug clearance leads to reduced area under the curve and shorter half-life, justifying considerations for higher doses and frequent redosing [102]. While some authors monitor B-cell depletion in order to decide on re-treatment, others have shown that monitoring of memory B-cells may be more important, since not all patients with B-cell recovery relapse [103].

Therapy with rituximab appears to be more useful when it is used to maintain remission of CNI-dependent disease, especially in patients with prolonged CNI use or its toxicity. Studies report high rates of complete or partial remission, reduced immunosuppressive drug requirement, and/or improvement in eGFR in patients treated with rituximab [99]. However, B-cell repopulation is followed by recurrence of relapses, leading to consideration of additional immunosuppression (e.g., with MMF) [96] or repeated doses of rituximab, either following relapses or upon B-cell repopulation as strategies to sustain remission. Given concerns regarding adverse events, the optimal dosing strategy remains unclear.

In an interesting small series by Basu et al., 10 out of 15 patients achieved complete and 5 achieved partial remission in SRNS patients when MMF was given after an initial lack of response to RTX; most patients had MCD [104]. Whether this protocol increases remission rates after RTX needs to be confirmed by future studies, however. Kamei et al. treated 10 patients with SRNS who were unresponsive to CNIs with RTX and pulse steroids and was able to achieve remission in 6 children [105]. Although this approach needs to be confirmed by a prospective, controlled study, it may be that a combination of RTX with other immunosuppressants (MMF, steroids or others) may be successful in some patients with SRNS.

Ofatumumab

This fully humanized anti-CD20 monoclonal antibody induced remission in anecdotal cases of CNI-

and rituximab-refractory SRNS and rituximab- and plasmapheresis-refractory posttransplant recurrent FSGS. Basu administered ofatumumab to two patients with MCD and three patients with FSGS [106]. All received multiple medications before this intervention, including two courses of RTX. After ofatumumab, improvement of proteinuria and an increase in the serum albumin levels from 1.2 g/dl to 3.1 g/dl within 6 weeks was noted. Hypoalbuminemia resolved after the third dose and remission was achieved after six doses. No serious side effects were reported [106]. However, a subsequent placebo-controlled RCT was terminated prematurely for futility after none of 13 patients with steroid- and CNI-refractory SRNS administered one dose of ofatumumab (1500 mg/1.73 m^2 IV) showed response [107]. Ofatumumab, however, may be an alternative antiCD20 agent in patients with intolerance reactions to rituximab.

Cyclophosphamide

Cyclophosphamide is an alkylating agent acting on B- and T-cells; while it has been widely used in steroid-sensitive nephrotic syndrome results in SRNS did not show a benefit when compared to oral steroids alone [70, 108]; therefore, oral cyclophosphamide is no longer recommended for management of SRNS.

Intravenous cyclophosphamide has been shown to be more effective that oral administration in in two Indian studies; rates of complete remission were 14.8–29.2% of patients [109, 110]. Another RCT comparing IV to oral cyclophosphamide in 50 children showed early remission (60% vs. 52%) followed by disease relapses in the majority; adverse effects were common (36% vs. 20%) [111]. Comparing intravenous cyclophosphamide to CNI, results of two multicenter RCTs indicate that therapy with IV cyclophosphamide is associated with significantly lower rates of remission than CNI (RR 0.5; 95% CI 0.4–0.7) [70]. One study showed that therapy with cyclosporine (150 mg/m^2/day) versus IV cyclophosphamide (500 mg/m^2 q monthly for 7 doses) was associated with remission in 9 of 15 (60%) versus 3 of 15 (20%) at 6 months [112]. Another study of 131 patients, found 6-month complete and partial remission rate of 45.9% with IV cyclophosphamide and 82.5% with tacrolimus; the rate of complete remission was 14.8% and 52.4%, respectively [110]. Given the unsatisfactory response to therapy with IV cyclophosphamide, the use of this medication is considered only in patients with contraindication to CNI, and in regions where CNI are not available.

Combination of Immunosuppressive Agents

As treatment-resistant FSGS carries a high risk of progression into ESKF, attempts to achieve remission with other biological drugs and interventions are important. Every single patient achieving complete remission should be viewed a success. If no response with a single-drug approach cannot be achieved, combination of different drugs may be an option. For instance, information on 49 patients (4 case series) with CNI-refractory or CNI-dependent SRNS, summarized in a recent review, indicate that therapy with the combination of CNI and MMF might enable remission in 33 (67.3%) patients with CNI-refractory disease, without major adverse events [87]. Multi-therapy with rituximab, IV methylprednisolone pulses, and cyclosporine or MMF was associated with complete remission in six patients with CNI-refractory SRNS; however, redosing was associated with hypogammaglobulinemia [113]. Controlled studies with vigilance for infections should evaluate the safety and efficacy of strategies involving intense immunosuppression.

Novel Therapeutic Strategies

These include immunosuppressive and non-immunosuppressive approaches. Regarding immunosuppressive treatment, several agents have been studied in SRNS.

Anti-IL 2 Antibodies

In a pilot case–control study with five patients who were resistant to all available treatments, including RTX, Bonanni et al. administered anti-IL 2 antibodies, because IL2 blockade increases Treg lymphocytes; in experimental models, upregulation of Treg immunity improves nephrotic syndrome. Unfortunately, in their study, Bonanni did not ameliorate proteinuria, and in all five children, kidney disease had progressed after 2 years [114].

Abatacept

This fusion protein of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and immunoglobulin blocks the CD28:CD80/CD86 interaction, thus blunting the costimulatory signal for T-cell activation. Yu et al. demonstrated CD80 (B7-1) overexpression in podocytes in biopsies in primary and recurrent FSGS as well as remission of proteinuria in five patients with CNI-refractory SRNS or posttransplantation recurrence treated with abatacept (given at 10 mg/kg IV once, repeated every 15–30 days, if required and tolerated) [115]. However, subsequent studies have contested the findings of CD80 expression on podocytes as well as the efficacy of abatacept in inducing remission [116]. Currently, a new trial investigation of the role of abatacept is under way implementing a precision medicine-based approach [117].

Adalimumab

This monoclonal antibody that blocks tumor necrosis factor (TNF)-α was associated with partial remission in four out of ten patients with primary FSGS in the FONT-I study, a phase I trial [86]. However, none of seven patients randomized to receive adalimumab (at 24 mg/m^2 administered subcutaneously biweekly for 16 weeks) exhibited remission at 6 months in the FONT-II trial [118].

Fresolimumab

This monoclonal antibody targets transforming growth factor-β (TGF-β), overproduction of which is implicated in the pathogenesis of fibrosis in FSGS. While a phase I trial reported complete or partial remission in 3 of 16 adults with primary FSGS administered one IV dose at 1 mg/kg [86], none of the 26 patients randomized to receive a dose of fresolimumab (at 1 mg/kg or 4 mg/kg) in the subsequent phase II trial achieved remission, leading to trial closure for futility [119].

Adrenocorticotropic Hormone (ACTH)

While ACTH was replaced by prednisone as the primary therapy for nephrotic syndrome in 1960s, interest in this synthetic hormone was revived by uncontrolled studies demonstrating significant reduction in proteinuria in adult patients with glomerulonephritis treated with ACTH gel (80 U/1.73 m^2 twice weekly) or intramuscular administration of its analogue tetracosactide (1 mg weekly for 1 year) [120]. A prospective pilot study of 24 patients using the same dosing strategy reported full remission in 2 and partial remission in 5 of 15 adults with steroid-resistant FSGS [121]. The multicenter ATLANTIS RCT that randomized children with frequently relapsing steroid-sensitive disease to receive therapy with ACTH (80 U/1.73 m^2 twice weekly) for 6 months versus standard therapy, was terminated prematurely for futility after interim analysis in 31 patients [122]. ACTH is still promoted by some authors [123]; in experimental models, ACTH seems to be able to support podocyte recovery [124].

Non-immunosuppressive Strategies

Other recent studies also evaluated non-immunosuppressive interventions to treat SRNS.

Low-Density Lipoprotein (LDL) Apheresis

Lowering of lipid levels might improve macrophage function, reduce endothelial dysfunction, prevent direct lipotoxicity to the glomeruli, improve renal blood flow, and reduce inflammation. Information on LDL apheresis in 47 patients (17 children) with SRNS, including CNI-refractory and recurrent FSGS, was summarized recently [125]. Apheresis was performed using Liposorber® LA-15 or LA-40 filters (Kaneka Pharma America LLC), based on dextran sulfate cellulose adsorption, for median 12 (range 4–24) sessions over 9 (2–52) weeks, with or without concomitant use of steroids, cyclosporine, and/or statins. Complete and partial remission were reported in 19 (40.4%) and 14 (29.8%) patients, respectively. However, a prospective multicenter study on 17 patients (15 children) with drug-resistant primary or recurrent FSGS reported remission in only 2 of 4 patients at 3 months and 2 of 3 patients at 6 months after therapy

[126]. Apheresis carries risk of complications similar to other extracorporeal therapies, and the equipment is not marketed in several regions. In another pediatric study, seven patients with recurrence after transplantation were treated with steroids and LDL-A and all showed reduction of proteinuria or complete remission and demonstrated improvement in their GFR [127]. Taken together, LDL-apheresis might proof to be a valuable rescue treatment, although publication bias may be present; more studies are warranted.

Galactose

Anecdotal reports suggested that oral galactose binds to the serum "circulating" factor to inhibit its activity and reduce proteinuria in individual patients with refractory FSGS [9, 11, 128]. However, a subsequent trial of oral galactose (0.2 g/kg twice daily, max. 30 g daily) found no reduction in proteinuria in seven pediatric patients with SRNS, although glomerular permeability assay showed an improvement [129]. A phase II clinical trial reported partial remission in 2 of 7 patients with FSGS at 6 months of therapy with oral galactose [118]. Although the authors concluded that further studies using galactose should be performed and, for example, patients with earlier disease stages should be treated. Practical difficulties in using galactose include the need to ingest large oral doses and lack of ready availability.

Sparsentan

This is a dual-acting angiotensin receptor blocker (ARB) and highly selective endothelin type A receptor antagonist. A phase II blinded RCT (DUET) compared reduction in proteinuria following therapy with oral sparsentan (200 mg, 400 mg, or 800 mg, once daily) to oral irbesartan (300 mg, once daily) in patients with primary FSGS [130]. Therapy with sparsentan showed greater reduction in proteinuria (45% vs. 19%) and a higher (28% vs. 9%) proportion of patients achieved the partial remission end point (spot urine protein to creatinine ratio ≤ 1.5 g/g and $>40\%$ reduction from baseline) without significant adverse effects [130]. While results of another RCT (DUPLEX) are awaited, the agent appears promising as adjunctive therapy in SRNS.

Adjunctive Therapies

Prednisone on alternate days and RAAS inhibitors usually accompany all immunosuppressive strategies. Prednisone is initially given at 30–40 mg/m^2 (1–1.5 mg/kg) on alternate days and tapered to discontinue therapy 6–9 months later in those that enter remission [69]. Disease relapses are common, and usually steroid-sensitive; compliance and adequacy of therapy with CNI must be ensued in patients with frequent relapses.

Therapy with RAAS blockers (ACEI or ARB) is recommended for all patients with SRNS since it is associated with dose-dependent reduction in proteinuria, known to retard CKD progression via the TGF-ß pathway. It has been used in combination with indomethacin (chemical nephrectomy, especially in genetic forms of SRNS) [75]. Therapy is withheld in presence of hypovolemia and/or AKI. The potential future role of sparsentan, a dual-acting ARB and highly selective endothelin type A receptor antagonist that was shown to achieve greater reduction of proteinuria than irbesartan has been mentioned.

Supportive Care and Management of Complications

Principles of management of complications and components of supportive care will be covered elsewhere (see ▶ Chap. 14, "Steroid-Sensitive Nephrotic Syndrome").

Outcomes

Information on disease activity in SRNS on the long term is limited. Outcomes on medium-term range from sustained remission after CNI are discontinued, to a relapsing illness that requires switch to less toxic regimens [62, 79]. Pooled data from five large multicenter collaborative studies including 1107 patients with SRNS indicates complete remission in 26.7% [95% CI 24.2–29.4%] cases, partial remission in 18.4% [16.2–20.8%] cases, and nonresponse in 54.8% [51.9–57.7%] patients [67, 76, 101, 131], Table 2. The lower rates of remission as compared to randomized

Table 2 Rates of response and kidney failure in children with genetic and nongenetic forms of steroid-resistant nephrotic syndrome

Author, year [Reference]	Genetic cause, %[a]	Complete, partial remission		Kidney failure[b]	
		Nongenetic, N	Genetic, N	Nongenetic, N	Genetic, N
Trautmann, 2018	373/1554 (24%)	159/387	10/74	113/501$^{\wedge 1}$	116/241$^{\wedge 1}$
Landini, 2020	37/64 (57.8%)[c]	13/17	1/19[d]	3/6$^{\wedge 2}$	11/25$^{\wedge 2}$
Nagano, 2020	69/230 (30%)	41/158	2/37	79/158$^{\wedge 3}$	52/69$^{\wedge 3}$
Mason, 2020	81/271 (29.9%)	69/149	9/26	41/149$^{\wedge 4}$	16/26$^{\wedge 4}$
Total[e]	1086/3902 (27.8%)[e]	282/711 (39.7%)	22/156 (14.1%)	236/814 (29.0%)	195/361 (61.5%)
Genetic versus nongenetic disease		Odds ratio	95% confidence interval		P
Nonresponse		4.00	2.52, 6.51		<0.0001
Kidney failure		2.87	2.22, 3.72		<0.0001

Only reports based on next-generation sequencing; latest or largest report for units with multiple papers

[a] Congenital nephrotic syndrome not excluded, except by Trautmann et al.

[b] Numbers at [1]last follow-up; [2]at 10 years; or extrapolated from Kaplan–Meier analysis, at [3]last follow-up or at [4]10 years

[c] Includes 18 patients with phenocopies

[d] Excludes 18 patients with phenocopies

[e] Includes 526 of 1783 families tested by Sadowski et al. [6]

studies might reflect medium-term outcomes and real-world effectiveness of immunosuppressive therapies; however, large databases often lack accurate phenotyping and are limited by selection and information bias, risk of attrition, and confounding.

Multicenter observational cohorts demonstrate that etiology of disease and response to immunosuppression are the chief determinants of long-term outcome. Based on information from four studies, the odds ratio for nonresponse in patients with genetic versus nongenetic disease is estimated at 4.0 [95% CI 2.5, 6.5; $P < 0.0001$] and that of kidney failure at 2.9 [2.2, 3.7; <0.0001] [67, 76, 101, 132]. Among patients with undifferentiated or nongenetic SRNS, response to immunosuppression predicts kidney survival, with significantly higher risk of kidney failure in patients with persistent nonresponse. Pooling data from 5 cohorts, the rates of survival free of kidney failure at 10-year follow-up are estimated at 92.9% [95% CI 85.8–97.7%] in patients with complete remission, 72.6% [64.8–79.8%] in patients with partial remission, and 35.1% [22.2–49.3%] in patients with nonresponse [67, 76, 101, 131, 132].

Renal histology and type of steroid resistance might also impact outcomes. Patients with minimal change disease demonstrate higher rates of remission and better prognosis than those with FSGS [70]. Patients with FSGS tip lesion tend to have a favorable outcome, while collapsing glomerulopathy has the lowest remission rate and high risk of progression to kidney failure (see above). Information from large case series indicates that the majority of patients with late steroid resistance show minimal change disease on biopsy, usually respond satisfactorily to immunosuppression, and rarely progress to kidney failure. Secondary steroid-resistant patients had significantly higher complete response than those with genetic-testing-negative primary steroid resistance (43% vs. 23%; $P = 0.001$) [101]. However, data collated from RCTs indicates similar response to immunosuppression for patients with initial or late steroid resistance [70].

Overall, response to immunosuppression appears to be the most important determinant of long-term outcome in SRNS. Patients who continue to have disease relapses, persistent proteinuria or decline in renal function into adulthood require transition into care by “adult” nephrologists.

Transplantation

FSGS ranks third among the causes of kidney failure or transplantation in regional pediatric dialysis and transplant registries, accounting for 10–12% cases [in the North American Pediatric

Renal Trials and Collaborative Studies, Latin American Registry of pediatric renal transplantation and UK Renal registry]. Disease recurrence after transplantation, presumably secondary to the “circulating factor,” has been observed in the immediate posttransplantation period in 30–50% cases in retrospective series and is an important determinant of outcomes. Recent data from the NAPRTCS suggests a lower rate of 5-year allograft survival in patients with FSGS compared to other etiologies (74% vs. 87%) [133].

The rate of recurrence appears to be unchanged in the decades following changes in posttransplant immunosuppressive regimens. Summation of data from 5 recent large multicenter reports, including the UK National Registry of Rare Kidney Diseases [101], PodoNet registry [67], France [134], Italy [135], and the United States [133], indicates disease recurrence in 218 of 682 pediatric kidney allograft recipients, implying a recurrence rate of 32.0% (95% CI 28.6%, 35.6%). Recurrent FSGS is associated with allograft loss in 50–60% of cases that do not respond to changes in immunosuppression and therapeutic apheresis. Strategies targeting early detection and aggressive management of recurrent FSGS have improved outcomes to enable partial or complete remission of proteinuria in 60–75% cases [136]. Disease recurrence in the first allograft confers very high (>80%) risk of recurrence in subsequent allografts.

The chief risk factor for recurrence appears to be a diagnosis of nongenetic disease, reflected in difference in recurrence rates between patients with genetic versus nongenetic disease (<5% vs. 28–68% cases, respectively). Posttransplant proteinuria, affecting approximately 30% of patients with homozygous Fin-major mutations in *NPHS1*, is caused by a de novo glomerulopathy associated with the generation of anti-nephrin antibodies in response to the allograft nephrin, which represents an immunologically foreign molecule in patients lacking nephrin expression [137]. Despite anecdotal reports of recurrent disease in patients with *NPHS2* variations, the overall risk of posttransplant recurrence is low, similar to other forms of genetic SRNS that almost never recur.

Historically, the majority of patients with genetic disease might have been undiagnosed, due to lack of access to genetic testing and limited number of genes screened. Plausibly, the difference in risk of disease recurrence reported for patients with late (initial steroid sensitivity) versus initial steroid resistance (93% vs. 30%; odds ratio 30; 95% CI 6.6–135.9) [136] reflects the difference between nongenetic and genetic SRNS. Similarly, recurrence is more common in patients with native kidney biopsy finding of MCD versus FSGS (75% vs. 40%; adjusted odds ratio 5.6; 95% CI 1.3–23.7), and in older versus younger children (threshold age 6–9 years), categories that likely reflect the clinical phenotypes of initial steroid sensitivity (nongenetic disease) and genetic SRNS, respectively. However, the relationship with age is not definite; another study suggests higher risk in younger children. Other inconsistent risk factors are rapid progression to kidney failure (<3 years from disease onset), mesangial proliferation on native kidney biopsy, native kidney nephrectomy, Caucasian race, and living related (versus deceased) donor transplantation or HLA-AB match.

Evaluation for posttransplant recurrence of SRNS, and its management including preemptive approaches, is discussed elsewhere (see ▶ Chap. 82, “Recurrent Disease After Pediatric Kidney Transplantation”). Briefly, recent clinical practice recommendations advise attention to specific recipient and donor factors during that pretransplant evaluation.

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Membranous Nephropathy

16

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Abstract

Membranous nephropathy is a glomerular disease that mainly affects adults. In children, it can present starting from the first days of life, though it is more frequent in adolescents and young adults. Among glomerular disorders, it stands out as a disease whose pathogenesis in the last decade has been elucidated with the discovery of the driving antigen in the majority of cases, leading to the potential for simplified diagnosis and tailored therapeutic approaches. This chapter presents an overview on the different forms of membranous nephropathy that can affect children and on the advances in diagnosis and treatment that have been made in this exciting field.

Keywords

Lupus · Hepatitis B-podocyte · Neutral endopeptidase (NEP) · PLA2R · THSD7A · Exostosin 1/2 · NCAM1 · NELL-1 · Semaphorin 3B · Bovine serum albumin-Complement activation · Risk alleles · Genetics · Glucocorticoids · Cyclophosphamide · Calcineurin inhibitors · Anti-CD20 monoclonal antibodies · Anticoagulants

Introduction

Membranous nephropathy (MN) is histologically defined by the presence of deposits of immune complexes located on the outer aspect of the glomerular basement membrane (GBM). These deposits are electron dense by electron microscopy. By immunofluorescence (IF), they contain immunoglobulins G (IgG), the relevant antigens that have long eluded identification, and the membrane attack complex (MAC) of complement, thought to be the mediator of proteinuria. MN is the major cause of nephrotic syndrome (NS) in adults. In children, it is a rare disease and its diagnosis is currently made on a biopsy indicated because the atypical clinical presentation and/or outcome under steroid therapy does not fit with the diagnosis of minimal change disease, which is the most common cause of NS in this age range [76]. Clinical manifestations of MN include proteinuria, hematuria, overt NS, and in some cases kidney dysfunction.

There are few diseases in nephrology where pathophysiological advances have been as rapid and have had such a major impact on patient care as MN. At the time of writing this chapter, most antigens implicated in MN have been identified. It is remarkable that the first podocyte antigen ever identified in humans was neutral endopeptidase (NEP) in a rare subset of children with antenatal MN [24]. This discovery provided the proof of concept that in humans, the disease mechanism closely resembles the rat experimental model of Heymann nephritis [48] and paved the way for identification of the major antigen, PLA2R, in adult MN [5]. Pediatric cases also provided the first examples of food-induced MN caused by cationic bovine serum albumin [26]. Since then, additional antigens have been identified, providing clues to the etiology and invaluable tools for patient monitoring. We now know that these antigens are age-dependent, i.e., each antigen has a specific prevalence according to age. Identification of these antigens has reduced the number of “idiopathic” cases of MN and elucidated the link between certain diseases (cancer, SLE) and MN.

We have entered an era of therapeutic progress where biotherapies have a growing role. In adult MN, rituximab is now head-to-head with cyclophosphamide as first line of treatment in patients with MN and persisting NS [93]. Other agents modulating B cells, plasma cells, and complement are knocking at the door, and combination therapies should also be considered.

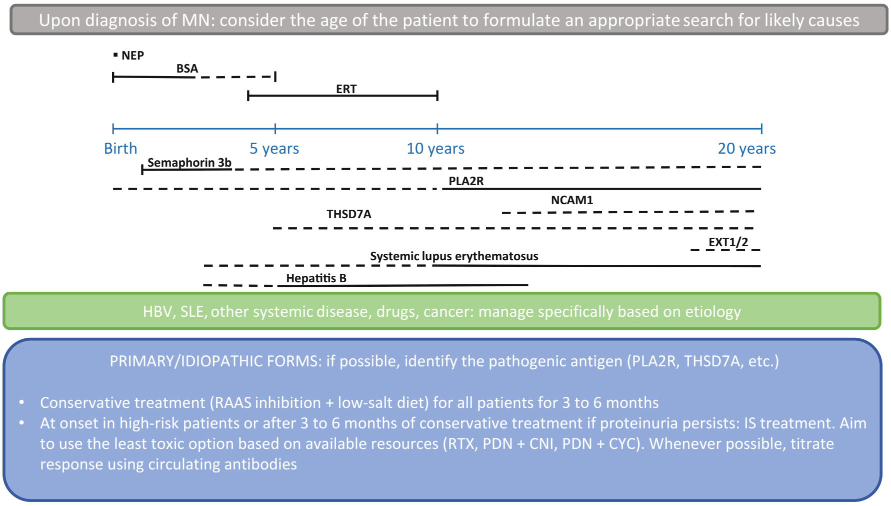
Incidence, Prevalence, and Etiology

MN in children is an infrequent histological diagnosis, especially compared to adults. Historically, while in adults its incidence was found to be 1.2/100.000/year, in children it was 0.1/100.000/year. However, its frequency as the underlying cause of NS steadily increases with age from about 1–2% at ages 1–5 years to about 5% at 10 years, about 10% at 15 years and roughly 18–22% at 18–20 years of age. As children with steroid-sensitive forms of NS are not routinely biopsied, some patients with forms of MN that respond to glucocorticoids may fail to be recognized.

In terms of gender, while in adults primary MN is more frequent in males compared to females, in children, the frequency is very similar between genders [76]. Chen et al. found a 6:7 male to female ratio in a US cohort of pediatric MN [16], and Liu et al. found 23 male and 21 female MN patients in a Chinese pediatric cohort [55]. Another study reports that MN was more frequently diagnosed in girls.

The etiology of MN varies with age. Figure 1 shows the most probable forms of MN at different ages.

In children <1–2 years of age, though exceptional, MN can be due to circulating antibodies of alloimmune origin, auto-antibodies to the phospholipase A2 receptor (PLA2R) transmitted by a mother with immunologically active MN [75], or semaphorin 3B [79]. In the congenital form, the mother carries a homozygous mutation in the *MME* gene (3q25.2) coding for neutral endopeptidase (NEP), a protein expressed on podocytes. Maternal antibodies against NEP are formed during pregnancy and pass the placental barrier during fetal life, leading to the presence in the offspring of MN due to alloimmunization with maternal antibodies [24]. Another early-onset



Upon diagnosis of MN: consider the age of the patient to formulate an appropriate search for likely causes

HBV, SLE, other systemic disease, drugs, cancer: manage specifically based on etiology

PRIMARY/IDIOPATHIC FORMS: if possible, identify the pathogenic antigen (PLA2R, THSD7A, etc.)

- Conservative treatment (RAAS inhibition + low-salt diet) for all patients for 3 to 6 months
- At onset in high-risk patients or after 3 to 6 months of conservative treatment if proteinuria persists: IS treatment. Aim to use the least toxic option based on available resources (RTX, PDN + CNI, PDN + CYC). Whenever possible, titrate response using circulating antibodies

Fig. 1 Suggested management of MN in childhood. Upon diagnosis of MN in child, usually by kidney biopsy but potentially in older children/adolescents by identification of positive anti-PLA2R circulating antibodies, a logical step-wise approach should be followed. First, based on the age of the child, the most likely causes must be identified (see text for details). Briefly, in neonates, the congenital NEP form must be suspected; in infants, the cBSA form; in children aged 1–3, the semaphorin 3B form; in children with a metabolic disease on recombinant human arylsulfatase B or alpha-glucosidase, the enzyme replacement therapy (ERT) form; in children living in HBV endemic areas particularly aged 5–7, HBV form; and in children > age 10–12, either SLE or primary forms driven by one of the known antigens, particularly PLA2R

form of MN due to an immune reaction, this time directed against a xenoantigen, is caused by IgG antibodies directed against cationic bovine serum albumin (cBSA). It presents in infants and small children (5 months–2.3 years of age in the initial disease description) fed with cow's milk. The subepithelial IgG deposits are immune complexes containing cationic bovine serum albumin [26]. Very recently, a primary form of MN due to anti-semaphorin 3B antibodies has been described, which can present also in children below the age of 2 [79].

In children >2 and below the age of 10–12 years, MN tends to be more frequently secondary. In endemic areas, hepatitis B is most frequently the cause, although many different infectious agents have been associated with. HBV-associated MN by horizontal transmission presents in younger children, usually at 5–7 years of age [11]. Vertical transmission from mother to child is also possible, and in this case, it presents in infancy. The clinical features of HBV-MN are variable; however, nephrotic syndrome is frequently observed, with a very marked (>80%) male predominance. As HBV vaccination becomes universally widespread, the incidence of this form is destined to decrease steeply.

In this age group and in adolescents, SLE is the other major cause of secondary MN. Class V lupus nephritis represents 10–12% of all forms of lupus nephritis in children. Other forms of secondary MN are listed in Table 1. Of note, extremely rare forms of alloimmune MN that can occur in this age group affect children with rare metabolic diseases such as Pompe disease and mucopolysaccharidosis type 6 requiring enzyme replacement therapy (ERT) with alpha-glucosidase and recombinant human arylsulfatase B (rhASB), respectively [27, 44].

Mostly but not exclusively in children aged >10–12 years and in adolescents, MN is due to

Table 1 Secondary forms of membranous nephropathy. (Modified from Valentini Chapter PN7)

Infection	Autoimmune and dysimmune	Drugs	Neoplasia	Alloimmune and other antigens	Other causes
• Hepatitis B in endemic areas • Hepatitis C • Malaria • Syphilis (congenital) • CMV, EBV • Tubercolosis • Bacterial endocarditis (Streptococcus, enterococcus) • Leprosy • Infestations (hydatid cyst, schistosomiasis, filariasis)	• SLE • IPEX • Graves disease, Hashimoto disease • Autoimmune hepatitis • Sarcoidosis • Ulcerative colitis, Crohn's disease, ankylosing spondylitis • Celiac disease • Type 1 diabetes • Sjogren, RA, SS, Mixed connective-tissue disease, dermatomyositis • Pemphigus • Fanconi syndrome due to anti-TBM antibodies • Idiopathic thrombocytopenia	• Gold, mercury • Infliximab, etanercept • Penicillamine • Tiopronin • Captopril • NSAIDs	• Ovarian • Neuroblastoma • Histiocytoma • Wilm's tumour • Gonadoblastoma	• Feto-maternal: MME mutation • Anti-BSA antibodies • Genetic: • ERT in MPS type 6 and Pompe	• De novo post renal Tx • Post BMT • Sickle cell disease • Primary biliary cirrhosis • IgA deficiency

circulating antibodies that recognize antigens expressed on the podocyte, mostly PLAR2 which accounts for the increase in MN prevalence in this age group and rarely THSD7A.

Of note, the incidence of MN is rising steadily in the last 10–15 years. A recent study performed in China shows that between 2004 and 2014, the adjusted odds for MN in adults rose 13% annually over the 11-year period, and that long-term exposure to high levels of pollution, quantified as fine particulate matter <2.5 μ, increased the risk of MN. Within the same study population, a subsequent study focused on children showed a rise in prevalence of MN from 3% of pediatric glomerular diseases in 2004 to 7% in 2014. The reason for this rise is unknown. However, it can be hypothesized that a genetic susceptibility (first hit) combined with a rise in exposure to fine particles due to pollution (second hit) may give rise to the disease. This is most likely not limited to China, as other reports have described a similar increase in India and Pakistan and in Turkey. The steeply declining rates of hepatitis B infection in China, Taiwan, and South Africa, due to national vaccination programs, make it much more likely that the rise in cases of MN is indeed pertaining to primary/idiopathic forms of the disease [76].

Pathophysiology

General Mechanisms

A great deal of our knowledge on the pathogenesis of MN was provided by a model established in 1959 by a pediatrician, Walter Heymann, who immunized rats with extracts of the proximal tubule [40]. This animal model relies on the active immune response of the rat against a brush border antigen and therefore is defined active Heymann nephritis (AHN). It was long hypothesized that the antibodies recognized a cross-reactive antigen present in the blood, thus forming circulating immune complexes that became deposited in the GBM. This hypothesis was contradicted several years later by studies performed in the passive Heymann nephritis (PHN) rat model. In the PHN model, a fraction from the brush border of rat proximal tubules was isolated and injected into sheep to produce antibodies. These antibodies induced MN upon injection to rats. When infused into isolated rat kidney ex vivo, i.e., in the absence of circulating antigen, they were shown to bind podocyte autoantigens thus forming in situ immune complexes that stained positive for IgG, C3, and C5b-9 [20, 88]. Later this antigen was identified by Kerjaschki et al. [47, 48] as the podocyte membrane protein megalin which is identical to the membrane receptor of low-density lipoproteins LRP2 with a MW of about 600 kDa.

Twenty years after megalin/LRP2, the first pathogenic human antigen, an enzyme called neutral endopeptidase (NEP), was identified in 2002 [24] in a case of neonatal MN. NEP is present on the podocytes and the brush border of the renal tubules. The mother had truncating mutations in the *MME* gene, leading to the absence of NEP. Consequently, anti-NEP antibodies were produced during pregnancy and were transferred to her fetus during the last trimester, inducing neonatal MN. Other four families with the same mechanism of disease have been identified [25, 91]. Importantly, production of anti-NEP IgG1 is necessary for the disease to develop, while infants born to mothers producing only IgG4 were not or weakly affected [25, 91]. This is due to the fact that IgG1, not IgG4, activates complement via the classical pathway and inhibit enzyme activity.

Target antigens of circulating antibodies can be classified as intrinsic or extrinsic whether they are produced by the patient or they are provided by external sources (food for instance). The immunopathological characteristics of those antigens and the immune response are described in Table 2. A schematic representation of the pathogenesis of MN is provided in Fig. 2.

Intrinsic Podocyte Antigens

The identification of target antigens in adult MN was unsuccessful for a long time. In 2009, Beck

Table 2 Laboratory investigations and pathology features of MN in children, based on antigen specificity

Podocyte antigen	MN presentation	Age	Serum	Tissue IgG subtype predominance	Unique tissue findings	Prevalence
NEP 1 (intrinsic)	Fetomaternal alloimmune MN	Newborns	Anti-NEP antibodies (in mother and newborn)	IgG1	Staining for NEP within subepithelial deposits	Rare
cBSA (extrinsic)	Xenoantigen immune MN	Infants and young children fed on cow's milk	1. Circulating cBSA 2. Anti-cBSA antibodies	IgG1 or IgG4	Staining for BSA within subepithelial deposits	Rare
PLA2R (intrinsic)	Primary MN	Adults Adolescents Young children Newborns	Anti-PLA2R antibodies	IgG4	Staining for PLA2R within subepithelial deposits	Adults and adolescents: ~70% of primary MN Young children: Uncommon Newborn: Rare, associated with anti PLA2R antibodies transfer across the placenta or by breast feeding
THSD7A (intrinsic)	Primary MN Associated with increased risk of malignancy	Adults Children	Anti-THSD7A antibodies	IgG4	Staining for THSD7A within subepithelial deposits	Adults: 3–5% of primary MN Children: Occurs but unknown prevalence
Exostosin 1,2 (intrinsic)	Lupus membranous nephropathy (class V)	Adults (young women)	Not detected	IgG1	1. Staining for EXT1 and EXT2 along the GBM 2. Tubulo-reticular inclusions	Adults: 16–30% of class V lupus nephritis, rare in primary MN Children: Not tested (youngest reported case: 17 yrs)
NELL-1 (intrinsic)	Primary MN or malignancy – Associated secondary MN	Adults	Anti-NELL-1 antibodies	IgG1	Incomplete staining for IgG and NELL-1 within subepithelial deposits	Adults: about 8% Children: Unknown
Semaphorin 3B (intrinsic)	Primary MN	Children, even 1–3 years of age, and young adults	Anti -semaphorin 3B antibodies	IgG1	1. Staining for semaphorin 3B within subepithelial deposits 2. Tubular basement	Children: 15% of non-lupus MN

(continued)

Table 2 (continued)

Podocyte antigen	MN presentation	Age	Serum	Tissue IgG subtype predominance	Unique tissue findings	Prevalence
					membrane deposits of IgG 3. Tubulo-reticular inclusions	
Neural cell adhesion molecule 1 (NCAM1) (intrinsic)	Lupus membranous nephropathy (class V) Primary MN	Adults Children	Anti-NCAM1 antibodies	Variable	1. Staining for NCAM1 within subepithelial deposits 2.Staining for IgA, IgM and C1q 3. Tubular basement membrane, mesangial and vascular immune deposits	Adults: 6.6% of class V lupus nephritis, rare in primary MN Children: Occurs but unknown prevalence

Abbreviations: *MN* membranous nephropathy, *NEP* neutral endopeptidase, *IgG* immunoglobulin, *cBSA* cationic bovine serum albumin, *PLA2R* phospholipase A2 receptor, *THSD7A* thrombospondin type-I domain-containing 7A, *NELL-1* neural epidermal growth factor-like 1, *LMN* lupus membranous nephropathy

et al. [5] used glycoproteins purified from glomerular extracts to detect antibodies that reacted only under non-reduced conditions with a 185-kDa protein in about 70% of samples from patients with “idiopathic” MN. This protein was identified by mass spectrometry (MS) as the M-type phospholipase A2 receptor (PLA2R).

In 2014, Tomas et al. [85] using similar methods identified a second target antigen, trombospondin type 1 domain-containing 7A (THSD7A), in a small subset of patients (3–5%). Both proteins induce an IgG4-predominant humoral immune response.

More recently, a major technological advance was achieved by [78] using laser microdissection of glomeruli followed by MS identification of trypsin-digested proteins. They started from kidney biopsies of MN patients double negative for PLA2R and THSD7A antigens. A major difference with the immunochemical methods previously employed is that these authors use paraffin-embedded biopsies, instead of sera, which allows identification of the antigen even in patients with immunologically inactive disease who have cleared the antibody from the circulation but not the antigen from the deposits.

Exostosin 1/exostosin 2 (EXT1/EXT2) are the first two proteins that were identified using this technology and subsequently detected by immunohistochemistry (IHC) along the GBM of patients with PLA2R negative MN. The first report by Sethi et al. describes 26 EXT1/EXT2-associated MN with features of secondary MN, including staining for IgA or IgM or both in addition to IgG (mostly IgG1). The majority of the patients (81%) exhibited clinical and biopsy findings suggesting autoimmune disease with positive serology like anti-nuclear antibody (ANA), anti-double stranded DNA, anti-SSA/SSB, or anti-ribonucleoprotein antibodies and biopsy that was positive for glomerular C1q deposition and tubuloreticular inclusion bodies.

In the validation cohort, EXT1/EXT2 stained positive in pure lupus membranous nephritis (LMN) (8/18 patients) but cases of lupus nephritis that did not have a membranous component were

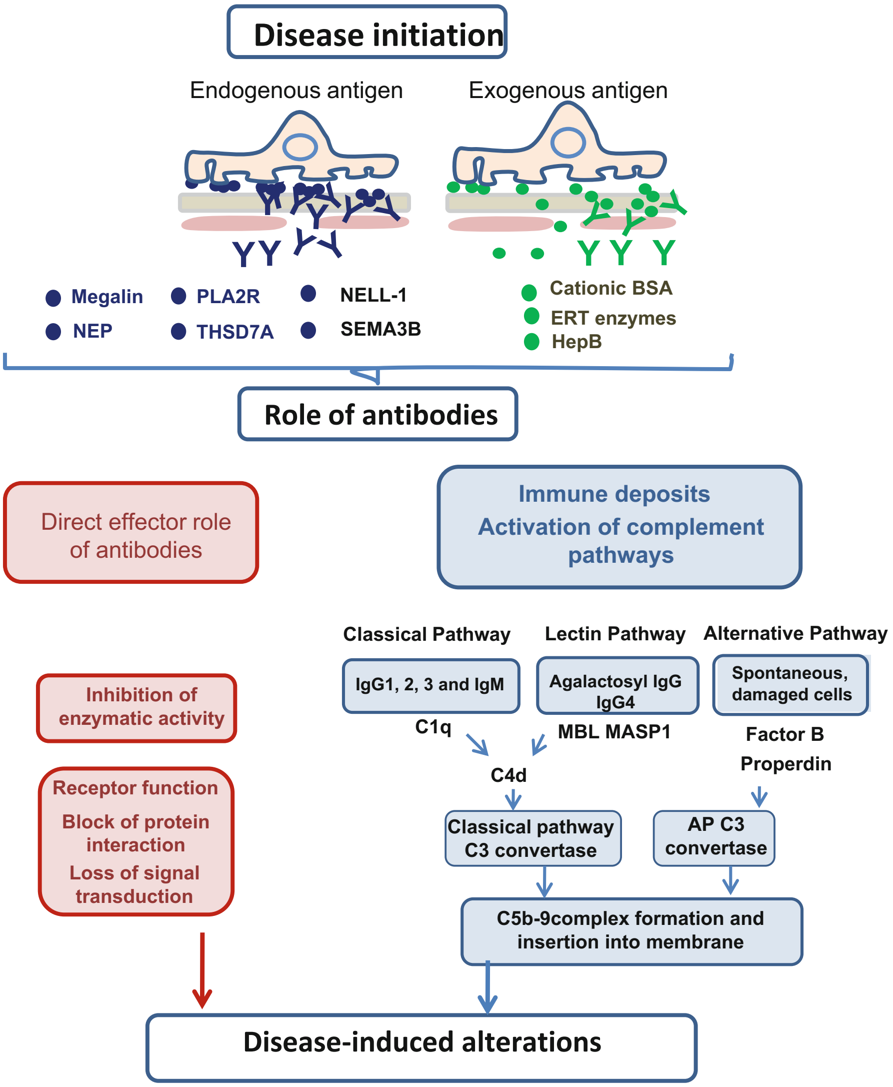

Disease initiation
Endogenous antigen
Exogenous antigen
Megalin
NEP
PLA2R
THSD7A
NELL-1
SEMA3B
Cationic BSA
ERT enzymes
HepB
Role of antibodies
Direct effector role of antibodies
Immune deposits Activation of complement pathways
Classical Pathway
Lectin Pathway
Alternative Pathway
IgG1, 2, 3 and IgM
Agalactosyl IgG IgG4
Spontaneous, damaged cells
C1q
MBL MASP1
Factor B
Properdin
C4d
Classical pathway C3 convertase
AP C3 convertase
C5b-9complex formation and insertion into membrane
Inhibition of enzymatic activity
Receptor function
Block of protein interaction
Loss of signal transduction
Disease-induced alterations

Fig. 2 Pathogenesis of MN

negative for EXT1/EXT2, suggesting that EXT1/EXT2 are specific to LMN. The authors were unable to demonstrate circulating anti-exostosin antibodies in 20 patients with EXT1/EXT2-associated MN, consequently EXT1/2 cannot be considered as yet as an antigen, but as a biomarker. In practice, EXT1/EXT2 staining may help identify LMN when the renal involvement precedes the development of extrarenal manifestations of SLE and when serology for active SLE is negative [78]. A study by Hanset et al. further specified that EXT1/EXT2-associated MN is more prevalent in young women with features of systemic autoimmunity [37]. Currently, the youngest patient described with EXT1/EXT2 is 17 [78].

Subsequent antigens identified by this method were NELL-1 [80], semaphorin 3B (SEMA3B), [79], and NCAM1 [14].

NELL-1 is a 90 kDa secreted protein produced mainly by osteoblasts. In the normal kidney, NELL-1 is detected in tubular cells but not in the glomerulus. NELL-1 becomes deposited in the extracellular matrix between podocytes and GBM and colocalizes with IgG within incomplete global or segmental deposits. The predominant humoral response is carried by IgG1, IgG3, and sometimes also IgG2, but not by IgG4. Importantly, a possible association with cancer was described in the French and Belgian validation cohorts and confirmed by Caza et al. in the largest series of 91 patients reported so far, in which one-third of patients had a cancer [15]. The youngest patient found with NELL-1 associated primary MN was a 30-year-old female. At this time, it is unknown if NELL-1 MN exists in children.

Semaphorin 3B (SEMA3B)-associated MN represents a unique type of primary MN that is more likely to be present in pediatric patients, particularly in very young patients with onset <2 years old [79]. Eleven cases have been identified, 9 in children. Confocal microscopy showed that both IgG and SEMA3B co-localized to the GBM (Fig. 3, panels I, J, and K). In 4 of the total 11 diagnosed cases, kidney biopsy also showed a unique finding of IgG deposits along the tubular basement membrane and tubuloreticular inclusions. Antibodies were detected in 4/4 patients with active disease but not in the one patient in clinical remission. They are predominantly IgG1 and react with the recombinant protein only in reduced state, which suggests that novel or cryptic epitopes are triggered by environmental factors or under pathophysiological conditions.

NCAM1 was most recently identified to be the target antigen in 6.6% of a large cohort of patients with LMN (14/212) and rarely positive (2%) in a smaller group of patients with primary MN (2/101) [14]. In the MLN group, all but one girl (12y) were adults with a mean age of 34y. Anti-NCAM1 antibodies have been detected in the serum of patient with MLN but it is yet unknown whether anti-NCAM1 antibodies correlate with disease activity. One MLN case with dual EXT and NCAM1 positivity is described.

It is unclear what triggers the immune system against intrinsic antigens such as PLA2R and THSD7A. In other cases, the antigen, although intrinsic, does not likely originate from the podocyte but may shed from other areas or from cancer and initiate an immunologic reaction and the formation of circulating immune complexes that subsequently become deposited in the GBM. This seems to be the case of most antigens recently described.

Extrinsic Podocyte Antigens

The first observation that a food antigen could be responsible for MN was reported in children with cationic bovine serum albumin (BSA)-related MN [26]. Again, this observation was inspired by an experimental model described by Adler et al. and Border et al. [1, 13]. Some patients, mostly infants and young children (5 months–2.3 years of age in the initial disease description) with classic histological findings of MN were found to have high serum titers of anti-BSA antibodies reacting with one peptide region of BSA but not with human serum albumin. Biopsies showed subepithelial IgG deposits containing cationic BSA. Change in BSA basic electric charge may derive from industrial processing of milk or from intestinal microbiota. The immaturity of intestinal walls in very small children fed with cow's milk may allow small amounts of this dietary antigen into the circulation, leading to formation of anti-cationic BSA circulating antibodies. In the kidney, due to its positive charge, the cationic BSA antigen is attracted to the negatively charged GBM and gives rise to immune complexes in the subepithelial region [26].

An exceptional form of MN has been described in children affected with rare metabolic diseases such as mucopolysaccharidosis type 6 and Pompe disease, which are due to a genetic mutation causing the lack of a specific enzyme. These children can therefore benefit from enzyme replacement therapy (ERT). However, due to the absence of this enzyme in the patient, ERT proteins over time can become potential extrinsic antigens that trigger immunization [27, 44].

MN can develop **actively** when the immune system is triggered against an antigen intrinsic or extrinsic to the podocyte. Infrequently, it can also develop **passively** as in the cases of antenatal

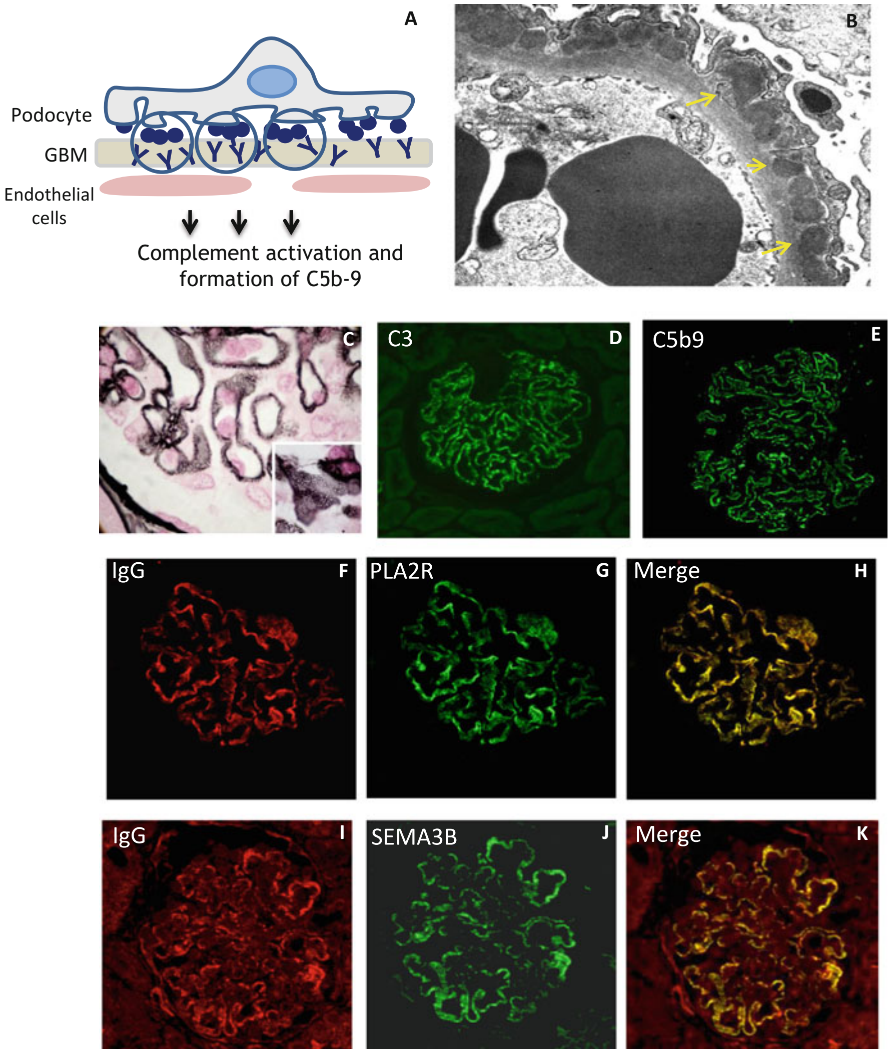


Fig. 3 Common histopathological pattern on kidney biopsy from MN patients. Panel A: Schematic representation of the mechanism of damage of the glomerular filtration barrier leading to proteinuria in MN. Blue solid circles represent the antigen at the podocyte surface, then contained with the bound IgG antibody (Y) coming from the blood in the immune complexes which are trapped in the subepithelial space. GBM = glomerular basement membrane. Panel B: Electron microscopy of a kidney biopsy of a patient with MN, the yellow arrows show the dense subepithelial immune complex deposits. Panel C: Silver stain of a kidney biopsy; by light microscopy, the thickening of the GBM is clearly visible with numerous spikes formed by expansion of the GBM with the appearance of numerous pinpoint "holes" in tangential sections, indicating deposits that did not stain with the silver stain (inset). Panels D and E: IF of C3 and C5b9 in a kidney biopsy of a patient with MN, showing staining along the GBM. Panels F–H: IF in a kidney biopsy of a patient with PLA2R-associated MN, showing IF positivity for IgG along the GBM in red (panel F), IF positivity for PLA2R along the GBM in green (panel G), and co-localization of the two in yellow (panel H). Panels I–K: IF in a kidney biopsy of a patient with semaphorin 3B (SEMA3B)-associated MN, showing IF positivity for IgG along the GBM in red (panel I), IF positivity for SEMA3B along the GBM in green (panel J), and co-localization of the two in yellow (panel K)

alloimmune MN associated with alloantibodies to neutral endopeptidase or transmission of anti-PLA2R antibodies from a mother to her fetus through the placenta and by breast milk [24, 75]. These passively developed cases of MN will recover when the route of transmission is removed.

Complement Activation and Podocyte Injury

The formation of immune complexes in subepithelial sites is central to MN resulting in complement activation identified by immunohistochemistry and more recently by MS [71]. Yet, the main deposited subclass is IgG4 that only weakly, if at all, activates complement pathways except in case of altered glycosylation. The amount of C1q is very low or undetectable in immune deposits of primary MN, indicating that activation of the classical pathway is not the main pathogenic mechanism. The presence of C4d in deposits in the absence of activation of the classical pathway suggests activation of the MBL pathway. Hayashi et al. found that the staining intensity of MBL correlated with the IgG4 staining intensity [38]. There is strong evidence that abnormally glycosylated IgG can activate the MBL pathway [61] and that PLA2R IgG4 can bind MBL [36, 96]. Glomerular deposition of factor B and properdin found in biopsies from MN patients provides evidence that both the alternative and lectin pathway can be involved. Cases of MN with MBL and ficolin deficiency have been reported, suggesting that formation of C5b-9 in those cases resulted mainly from hyperactivation of alternative pathway [3]. Moreover, MN was reported in a patient with C4 deficiency in which the classical and lectin pathways are not functional [53]. In contrast to PLA2R MN, the neonatal NEP form and NELL-1 and SEMA3B MN are mainly associated with IgG1, which activates the classical pathway, and C1q is found deposited in glomeruli in NEP and SEMA3B MN.

Complement activation and formation of the C5b-9 membrane attack complex (MAC) have major role in sublethal podocyte injury activating different mediators, such as phospholipases, protein kinases, cyclooxygenases, transcription factors, and cytokines. These signals influence podocyte cell metabolic pathways, structure and function of key cytoskeletal proteins and slit diaphragm, and DNA integrity [63, 82]. The injured podocytes produce new extracellular matrix. In addition to forming immune complexes, anti-podocyte antibodies may directly alter podocyte biology [72].

Gene Polymorphisms and Risk of Primary MN

MN is not a typical hereditary disease in Mendelian terms but there is growing evidence to support a strong genetic component. The initial genome-wide association (GWAS) study in 3 European cohorts including 556 patients of white ancestry found that primary MN in adults was strongly correlated with risk alleles, one in a noncoding single nucleotide polymorphism (SNP) within the *PLA2R1* gene (rs4664308) and another in the *HLA-DQA1* gene (rs2187668), a member of the HLA class II [81]. In individuals who were homozygous for both risk alleles, the risk of having MN was nearly an 80-fold as compared to individuals who had neither risk allele.

The same strong correlation between risk alleles (SNPs) of *PLA2R1* and *HLA-DQA1* and the risk of developing MN was found in a large Chinese cohort of 2132 individuals, including 1112 patients with IMN and 1020 healthy controls [58]. The authors concluded that patients with both risk alleles confer >11 times the risk of developing MN. They also correlated the presence of risk alleles to PLA2R antibodies.

Two studies performed on Chinese population revealed different HLA class II risk alleles associated with increased risk of MN [22, 51] and proposed that HLA associations may differ between ethnicities. This was reinforced in the largest international genetic study of MN performed recently. The study involved East Asian cohorts (1632 biopsy specimen – diagnosed cases and 3209 controls) and European-ancestry cohorts (2150 biopsy specimen – diagnosed cases and 5829 controls) [95] and found ethnic differences in HLA locus associations, defining *DRB1*1501* as a major risk allele in East Asians, *DQA1*0501* in Europeans, and *DRB1*0301* in both ethnicities. The *PLA2R1* locus (rs17831251) represented the most strongly associated non-HLA signal. In addition, this study identified two previously unreported loci, *NFKB1* and *IRF4*, linked to inflammation pathways. GWAS loci explain 32% of disease risk in East Asians and 25% in Europeans.

Clinical Features and Laboratory Investigations

The clinical presentation of MN is characterized by a variable degree of proteinuria, which can be asymptomatic, detected on routine urinalysis or more intense and quite frequently (25–100% of cases in different reports) in the nephrotic range, leading to NS (hypoalbuminemia, edema, hyperlipidemia). However, the onset of clinically evident nephrosis is usually more gradual than what is seen in minimal change disease, and asymptomatic nephrotic-range proteinuria has been reported in 16–38% of cases [16, 87]. Compared to adults, relapses tend to be more frequent. Moreover, while in adults microscopic hematuria can rarely occur, in children, it is frequently present (up to 70% of cases) [16] especially in children of Asian ancestry where it is reported in 90% of cases [86], while gross hematuria is reported in up to 40% of patients [52, 86]. Renal function is usually preserved [16], and hypertension is uncommon, but may be present in up to 36% of children at onset [92]. Thromboembolic complications, a serious complication of MN in adults, tend to be less frequent in children [57]. These features, together with the frequent association with concomitant associated causes (primarily HBV in endemic areas and SLE), differentiate MN in children from MN in adults (Table 3). Of note, a consistent group of children will receive a diagnosis of MN after having given several treatments (4–8 weeks of glucocorticoids), as they will have been initially treated for NS and then biopsied due to steroid-resistance.

Screening for infections, primarily hepatitis B and C, CMV and EBV should be performed. Other infections should be investigated based on the presence of associated clinical features.

Autoantibodies, in particular ANA and anti-dsDNA, should be performed in all cases. However, in the presence of isolated lupus nephritis without systemic features, particularly when anti-nuclear antibodies are negative, discriminating lupus nephritis with features of MN (class V lupus nephritis) from a primary form of MN can be challenging. In these cases, staining for EXT1/EXT2 and NCAM1 may be of great clinical use in predicting SLE (see below).

If systemic features are present, these will guide the search for other secondary causes (neoplasia, other autoimmune conditions such as systemic lupus erythematosus, Sjogren syndrome, Crohn's disease). A detailed history should be

Table 3 Differences between adult and pediatric forms of MN. (Modified from Ayalon R and Beck LH Jr. Membranous nephropathy: not just a disease for adults. Pediatr Nephrol. 2015 Jan;30(1):31–9)

Classification	Pediatric MN	Adult MN
Proportion of NS that is MN	1–15% from 2 to 18 years of age	15–50% from 18 to 50 years of age
Proportion of MN that is primary/idiopathic	Minority	Majority
Proportion of primary MN that is PLA2R-associated	45% (esp. adolescents)	70–80%
Clinical features		
Male predominance	No	Yes
Hematuria	70–90%	30–50%
Thromboembolic events	<5%	10–20%
Frequently relapsing course	Frequent	Rare
Progressive kidney failure	<25%	30–40%
Pathology		
Mesangial deposits	Infrequent in primary MN/ Frequent in LMN	Infrequent in primary MN/ Frequent in LMN
Segmental distribution of deposits	Seen	Rare
Deposits along the TBM	Typical of primary forms due to semaphorin 3b Abs in children, even <2 years of age	Extremely rare, seen in young adults with semaphorin 3b Abs

taken pertaining food and any medication, as mercury intoxication and a variety of medications such as penicillamine, infliximab, etanercept, tiopronin, captopril, and NSAIDs can trigger MN (see Table 1).

Histopathology

MN is a pathological term for lesions characterized by the accumulation of subepithelial deposits. These deposits are formed in the subepithelial space between podocytes and the GBM (Fig. 3, panel A) where they appear as electron-dense deposits by electron microscopy (Fig. 3, panel B). By *light microscopy*, these deposits cause thickening of the GBM. They are often associated with the formation of extracellular matrix around the deposits causing erect projections (spikes) that are better seen on Jones' silver stain (Fig. 3, panel C).

Composition of the deposits can be analyzed by *immunofluorescence (IF)*. They contain the complement component C3 (Fig. 3, panel D) and the complement terminal complex C5b-9 (Fig. 3, panel E) which contrasts with the quasi-absence of inflammatory cells in the glomerulus (most likely because complement activation occurs beyond the GBM in the subepithelial space). The deposits result in a fine granular pattern of polyclonal IgG staining along the capillary walls (Fig. 3, panels F and I). In some cases, deposits are segmental or global but incomplete, which suggests NELL1-associated MN although no pediatric case has been yet identified [15, 49].

Diagnosis of PLA2R-related MN can be established by IF (Fig. 3, panel G) or immunohistochemistry on paraffin sections stained with the relevant antibody. In forms of PLA2R-negative MN, staining for the other candidate antigens is recommended whenever possible to allow accurate diagnosis and therefore optimal management and monitoring (Fig. 3, panel J shows an example for semaphorin 3b). Antigen staining co-localizes with the polyclonal IgG staining (Fig. 3, panels H and K). IgG subclass determination is also relevant, as PLA2R-related forms have predominantly IgG4 while most cases associated with the recently identified antigens/biomarkers (EXT1/2, NCAM1, NELL1, and SEMA3B) have IgG1, and this determines downstream complement activation as detailed above.

MN can be divided into five stages by *electron microscopy* based on the position and radiolucency of the deposits. Initially, there were four stages 1–4; however, recently stage 0 has been added: Stage 1 – small and sparse subepithelial deposits without spikes or thickening of the GBM; Stage 2 – more extensive subepithelial deposits separated by projections of newly formed matrix material (spikes) between the deposits with thickening of the GBM; Stage 3 – deposits completely surrounded by basement membrane (intramembranous deposits); Stage 4 – deposits undergo absorption and become electron-lucent; and Stage 0 – no electron-dense deposits. This indicates a very early disease where LM appears normal but IF shows presence of IgG ± C3 staining in a capillary-loop pattern. This can be seen in recurrent MN posttransplantation by protocol biopsy. Of note, this staging system does not correlate with the clinical presentation (e.g., degree of proteinuria or prognosis).

Diagnosis, Serology, and Kidney Biopsy Staining

The diagnosis of MN has changed substantially in the last decade with the growing identification of podocyte antigens and associated biomarkers. Table 2 summarizes all the currently known antigens/biomarkers involved in MN and includes the MN category, the age group in which the antigen has been described, the serum finding and tissue staining for diagnosis, and prevalence based on published data. While ELISA and indirect immunofluorescence (IIF) assay for PLA2R-associated MN and IIF for THSD7A-associated MN have been developed commercially for detection and quantification of serum antibodies making diagnosis accessible to clinicians, for the other antigens this is not yet available. However, tissue staining for the latter antigens should be performed whenever possible.

Figure 1 represents the most likely forms of MN at different ages. Based on the prevalence of PLA2R-associated MN >10–12 years of age, it is reasonable to suggest performing an anti-PLA2R ELISA before a kidney biopsy in an adolescent with atypical presentation or steroid-resistant proteinuria (see below). In cases with a positive ELISA (>20 RU/ml), a biopsy is not required in the absence of deterioration of renal function and/or suspicion of a secondary cause [12].

The majority of MN cases can now be classified according to antigenic specificity (Table 2). In addition, they can be classified according to the cause. MN can be termed **primary** when the disease is associated serologically with antibodies to a known podocyte antigen. The term **idiopathic** MN is referred to cases in which the target antigen is yet unknown and serum antibodies are not recognizable and the disease does not appear to be secondary. **Secondary** MN is identified when the histologic lesion of MN can be associated with other systemic illness or exposures and the management relies primarily on treatment of the underlying cause of disease. However, the distinction between **primary** and **secondary** forms is somewhat artificial because finding specific antibodies or antigen staining in tissue does not rule out a secondary cause. On the contrary, finding EXT1/2 or NCAM1 suggests the diagnosis of MLN, finding THSD7A and NELL-1 suggests some forms of cancer in adults, and PLA2R positivity can coexist with hepatitis B, sarcoidosis, and cancer. One also should keep in mind that all **primary** forms have a cause triggering the immune response. The term **idiopathic** MN will become obsolete within a few years when all the antigens will have been identified.

Operationally, the discrimination of **primary** from **secondary** MN is currently justified because secondary forms may benefit from targeted treatment (i.e., SLE, cancer or hepatitis B). On the other hand, knowing the involved antigen may guide treatment (i.e., interrupting cow's milk or ERT) and allow accurate disease monitoring (i.e., anti PLA2R antibody titers, see below).

PLA2R-associated MN

M-type phospholipase A2 receptor (PLA2R) is the major antigenic target in adult primary MN. Circulating autoantibodies to PLA2R are found in 70% of cases [5].

The high prevalence and almost 100% specificity of anti-PLA2R autoantibodies in adult primary MN have given rise to the development of two FDA-approved serological tests for diagnosis and monitoring of disease activity. The first is an ELISA that gives a range of titers from less than 2 up to 1500 RU/mL, the second is a cell-based IIF assay which is more subjective and semi-quantitative, but has a higher sensitivity for low titer antibodies. ELISA titers below 20 RU/ml are considered negative according to the manufacturer [56, 84].

The prevalence of anti-PLA2R in adolescent patients with primary MN has been suggested to be similar to adult patients (81.1% in 11 children aged 13–17 years), while it is rare in children below 10 years of age [98]. An observational cohort study from India, also focusing on adolescents (10–19 years old) with primary MN, found that 83% had PLA2R-associated disease, as evidenced by glomerular staining for PLA2R on biopsy (14/18) and/or seropositivity for anti-PLA2R (13/18). Moreover, the levels of anti-PLA2R were associated with clinical status, suggesting that anti-PLA2R titers could be used to monitor immunological disease activity in children and adolescents, as in adults [50].

PLA2R-associated MN can also be diagnosed at the kidney tissue level by demonstrating the presence of the antigen within the immune deposits. In PLA2R-associated MN, we expect a prominent fine granular peripheral capillary wall pattern (Fig. 2, panel G) that can be co-localized with IgG (Fig. 2, panel H) and C3. In other MN cases that are non-PLA2R-associated or in normal controls, the PLA2R staining will be faint and localized to the podocyte, being absent from the immune deposits.

Recently, several algorithms have been suggested for the clinical use of the autoantibodies and the tissue staining [12, 23]. In the majority of PLA2R-associated MN with proteinuria, serum anti PLA2R antibody titers by ELISA and tissue PLA2R staining by IF of the immune deposits in the kidney biopsy are both positive. However, in some situations, the biopsy stains positive for PLA2R while the serum is negative for the antibodies. This situation more commonly arises at recovering disease, after the decline and disappearance of circulating antibodies, and explains the time that is required between immunologic resolution and clinical recovery. However, this same situation can rarely happen very early in the disease when the kidney is acting as a "sink" and is binding the majority of the autoantibody from the circulation [89]. This latter scenario often "seroconverts" to a positive anti-PLA2R status with time. Hence, in case of seronegativity for anti-PLA2R with positive PLA2R IF, close monitoring of proteinuria and serum antibody is necessary to distinguish between these two possibilities. This will allow to distinguish patients who are most likely to benefit from treatment from patients who might undergo spontaneous remission prior to treatment.

PLA2R-associated MN can recur after kidney transplantation. Kidney failure from MN in the pediatric population is rare but more frequent in adults. A few studies in the adult population suggest that higher levels of anti-PLA2R antibodies at the time of kidney transplantation increases the risk of recurrent MN [35]. This indicates that anti PLA2R antibodies may be pathogenic and suggests to delay transplantation and/or provide immunosuppressive treatment to allow transplantation when anti-PLA2R antibodies are no longer detectable.

Thrombospondin Type-1 Domain-Containing 7A (THSD7A)-Associated MN

THSD7A is the second antigen to be identified in primary MN. TSHD7A is much less prevalent than PLA2R, accounting for approximately 3–5% of adult patients with primary MN. THSD7A occurs in children but its prevalence in the pediatric population is unknown. Zaghrini and colleagues reported on a 4-year-old with anti-THSD7A antibodies that responded clinically and immunologically (antibody decline) to treatment with rituximab [97]. Similar to PLA2R, a simple IIF blood test for anti THSD7A may allow the diagnosis of primary MN. In addition, the presence of THSD7A antigen within immune deposits can be assessed using commercial antibodies to THSD7A. Commercial assays for anti THSD7A autoantibodies by ELISA are in the pipeline.

Although rare, double positivity to both autoantibodies and antigens to PLA2R and THSD7A has been described. Zaghrini et al. reported on a cohort of 49 MN adult patients positive to THSD7A (prevalence of 2.8% of primary MN) of whom 8 were also positive to PLA2R [97].

In the adult, positive staining for THSD7A can coexist with malignancy [43]. Hanset et al. found 50% solid organ malignancy in six patients with TSHD7A-associated MN. The three patients with tumor-associated MN had partial or complete remissions following treatment for their tumors [37]. Therefore, in adult patients with THSD7A-associated MN, aggressive screening for cancer should be pursued [8, 23, 65]. However, association with malignancy has not been described yet in children.

Prognostic Indicators and Treatment

The therapeutic approach to a child with MN should focus on the clinical condition and age of the patient and on the disease subtype (Fig. 1). Secondary forms, most frequently identified by screening for hepatitis B, C, and for ANA and anti-dsDNA antibodies and systemic features for SLE, must be approached by managing the underlying cause. For the correct management of lupus nephritis, see chap. Lupus nephritis. For the best approach to other secondary forms, see the specific section below. Due to its low prevalence in the pediatric population, evidence from randomized controlled trials enrolling children with MN are lacking. Therefore, it is particularly important that children with such a rare and potentially difficult condition be managed by experts in pediatric nephrology, using a personalized approach. Treatment of a patient with asymptomatic proteinuria should differ from that of a child with steroid-resistant nephrotic syndrome, weighing the risks against the benefits. The therapies to be evaluated range from supportive therapies to immunosuppressive therapies with potentially greater side effects.

The natural history of the disease in children and its long-term outcome is unclear, and it is not evident whether studies performed in an adult population, with a much higher incidence of comorbidities and a much lower life expectancy, can be extrapolated to children. While in adults the rate of spontaneous remission is reported to be between 30% and 40% [67], and conversely approximately one-third of patients described in historic cohorts progressed to terminal renal failure [34], in children similar historic cohorts (reviewed in [62]) and more recent studies [92] seem to suggest a more benign prognosis compared to adults. However, about 20% of pediatric cases reach end-stage renal disease [16, 52].

In children, predictors of outcome are not well defined; however, overall, some evidence indicates that nephrotic syndrome at onset [59], degree of proteinuria, hypertension, and interstitial fibrosis on the kidney biopsy may be correlated with a more severe outcome [16, 92].

In adults with primary PLA2R-associated MN, low/negative serum titers of circulating anti-PLA2R antibodies have been found to be positive prognostic factors and to predict spontaneous remission [42, 94]. The prognostic value of circulating antibody titers has been shown also for anti-THSD7A, and it appears likely to be of use in monitoring all primary forms of MN. Also in children with primary anti-PLA2R-associated MN, therefore, it is reasonable to assume that monitoring anti-PLA2R antibody titers can also be of clinical relevance in guiding therapeutic decisions and predicting spontaneous remission [2]. Though the availability of these assays may be limited to pediatric nephrologists especially in certain settings, their use should be implemented as widely as possible moving forward to avoid overexposing patients to potentially toxic immunosuppressive therapy once immunological remission is achieved. Drop in proteinuria may be seen long after (months to years) the decline and disappearance of anti-PLA2R antibodies. This can be explained by the time required for the kidney tissue to clear the deposits and to regenerate [6, 7].

Conservative Treatment

All children with significant proteinuria (>0.2 g/day or 0.2 g/g urine protein/creatinine ratio) should receive conservative treatment with ACE-inhibitor or angiotensin-receptor blocker at maximal tolerated dose. Evidence from adult studies [67] suggests that spontaneous remission was more frequent with lower baseline proteinuria. In this study, 80% of patients in spontaneous remission group were on ACEi/ARB compared with the group that did not attain spontaneous remission, in which only 60% were on ACEi/ARB. Dietary salt restriction is also necessary. In case of hypertension, the use of RAAS inhibition can have the dual benefit of anti-proteinuric and anti-hypertensive effect.

In adults, current guidelines [33, 93] suggest to employ antiproteinuric therapy in all MN patients, and to “wait and see” without adding immunosuppression for 6 months (so-called “wait and see” period) unless the patient has persistent nephrotic-range proteinuria (>4 g/day), reduced renal function, and/or additional risk factors for disease progression and/or serious complications (i.e., thrombosis). Immunosuppression is introduced in high/very high-risk adults at onset and subsequently if the patient fulfils the criteria for at least moderate risk children and proteinuria remains > 4g/day. In children, frequently upon diagnosis made by kidney biopsy, the child has already received some weeks of glucocorticoid therapy. Even in cases where the kidney biopsy precedes all therapeutic intervention, despite the reassuring historic data on outcome of MN in children, pediatric nephrologists tend to have a more proactive approach for the treatment of this disease with immunosuppression than adult nephrologists. Unless proteinuria is very low, immunosuppressive therapy is often used early after diagnosis. No evidence exists to inform this decision, which must take into account the age, general conditions, comorbidities, and wishes of the patient or of the family. The choice of the optimal immunosuppressive agent must be tailored to the patient, but substantial changes in the recommended therapeutic approach in adults, due to the completion of large studies, must be taken into account also in the management of children.

Glucocorticoids

Currently, no clinical trials evaluating the use of prednisone or equivalents in idiopathic MN and in children exist. Evaluating the effectiveness of this approach is made difficult by the fact that quite often a kidney biopsy diagnosing MN in children is performed for steroid-resistant nephrotic syndrome, after the patient has already received 4–6 weeks of full-dose oral prednisone and in some cases 3 methylprednisolone i.v. pulses. Therefore, children with MN who respond well to treatment with oral glucocorticoids may never be biopsied and therefore identified. Other pediatric patients, most likely due to older age at onset of proteinuria, will receive a diagnosis by kidney biopsy without previous glucocorticoid treatment. Moreover, in contrast to steroid-sensitive forms of nephrotic syndrome, which respond to glucocorticoids in days to weeks, MN is a disease that resolves much more gradually, and complete remission either spontaneous or induced by therapy normally requires months to years [42, 67]. In adults, evidence for the effectiveness of glucocorticoid monotherapy at disease onset does not support its use [18, 93]. In children, there is little evidence for the use of steroid monotherapy, although some small case series suggest that this approach may be effective. Valentini et al. demonstrated in a small series of idiopathic MN that, while 50% of the cases do not respond to steroids, there was complete or partial response in the remainder [87]. Similarly, in a series of 75 pediatric patients who underwent kidney biopsy for a non-MCD-like picture, 11 (14.7%) had MN. Of these, 8 were steroid sensitive and 3 were resistant [2]. Therefore, most children with intense or nephrotic-range proteinuria and MN receive at least 4 weeks of oral prednisone/prednisolone at doses used for steroid-sensitive nephrotic syndrome, followed by a tapering on alternate days for a total of at least 8–12 weeks. A second-line immunosuppressive treatment, most frequently a calcineurin inhibitor or rituximab, is frequently added.

Cyclophosphamide

Alkylating agents, mainly cyclophosphamide (CyC), have been extensively studied in the adult population with MN. While available evidence does not support their use as monotherapy [29], regimens employing i.v. methylprednisolone pulses followed by oral prednisone for 30 days followed by oral chlorambucil/cyclophosphamide for 30 days on alternate months for a total of 6 months have been shown to be clearly superior to conservative therapy in adults with MN and nephrotic syndrome. Cyclophosphamide was preferable to chlorambucil in terms of safety (reviewed in [18]).

In children, a small case series using cyclophosphamide for 12 weeks in addition to alternate day oral prednisone showed a good response [87]. However, cyclophosphamide, especially in young patients and females, is associated with two dangerous side effects: increased risk of neoplasia (bladder and hematologic) and gonadal dysfunction [46, 74]. In a recent retrospective investigation of treatment strategies employed for children and adults with MN in North America, 0% of children received cyclophosphamide in the first 6 months from diagnosis, and only 1 (3%) subsequently [64]. Given the concern for gonadal suppression and other adverse effects, it is our opinion that alkylating agents should be avoided in most, if not all, cases of pediatric MN if calcineurin inhibitors or rituximab are available as treatment options.

Calcineurin Inhibitors

Calcineurin inhibitors in combination with glucocorticoids have been used extensively in adult MN. There is some evidence from adult studies suggesting that tacrolimus may be more effective than cyclosporin A [68].

Several studies including randomized-controlled trials have shown that remission of proteinuria in tacrolimus-based therapy was comparable to that of cyclophosphamide and glucocorticoid [17, 39, 69]. These results, added to the more favorable safety profile of calcineurin inhibitors (CNI) compared to alkylating agents and to the greater familiarity that pediatric nephrologists have with CNIs, have led to their use being relatively frequent in children with MN. However, evidence of their effectiveness is tempered by the repeated finding of about 50% relapse of proteinuria following their discontinuation in children with MN. When initiating a CNI for MN, prednisone is usually given at standard doses used in steroid-sensitive nephrotic syndrome (60 mg/m2/day) for 4–6 weeks total (i.e., taking into consideration the dose taken pre-kidney biopsy in those children who were biopsied for steroid resistance), then tapered and discontinued over 3–6 months. CNIs are given for at least 6–12 months and need to be tapered very gradually to prevent relapse. Monitoring of blood doses is useful, and if no improvement in terms of proteinuria is seen after 6 months, CNIs should be discontinued and an alternative approach attempted.

Anti-CD 20 Monoclonal Antibody: Rituximab
The discovery of a number of circulating antibodies forming the immune complexes found in the subepithelial space in primary MN, especially anti-PLA2R, but also anti-THSD7A, and more recently and relevantly to the pediatric population, anti-SEMA3B has been a major progress of nephrology in the last decade. This finding has clearly provided a pathophysiological rationale for the use of B-cell depleting agents in primary MN, in order to reduce circulating antibodies and therefore the formation of the immune complexes that leads to damage of the glomerular filtration barrier and to proteinuria. In particular, rituximab has been shown to be effective in at least two-thirds of adults with these forms on MN. The GEMRITUX trial was a multicentric randomized controlled trial performed in France, in which 77 patients with normal renal function were randomized to either rituximab (375 mg/m2 on days 1 and 8) or continuation of non-immunosuppressive anti-proteinuric treatment (NIAT) after a 6-month lead-in period of NIAT in each arm [23]. In the follow-up period after the first 6 months, in which the clinical difference was not significant, the remission rate in terms of proteinuria was of 65% in the rituximab group, compared to 34% in the control group. When assessing anti-PLA2R antibody titers at 6 months, they were significantly reduced in the rituximab group. This confirms that a reduction in antibody titers precedes the reduction in proteinuria and suggest that monitoring of titers can be used to predict the need for a repeat infusion. In terms of number of rituximab doses, Cravedi et al. [21] showed that a single dose, if capable of inducing total (<1% of circulating lymphocytes) B cell depletion at 7 days, is as effective as multiple doses in inducing remission of proteinuria. A retrospective cohort study comparing the use of rituximab with this approach to the use of glucocorticoids and oral cyclophosphamide according to a well-defined standardized protocol showed at 5 years no difference in terms of achieving complete remission, with a better safety profile in the rituximab-treated group [90]. More recently, the MENTOR trial results were published [31]. This randomized controlled study conducted in North America compared rituximab to cyclosporine, including 130 patients with at least 5 g/day of proteinuria. Patients were treated with rituximab (2 infusions, 1000 mg each, 14 days apart, repeated at 6 months in case of partial response) or cyclosporine (at a dose of 3.5 mg per kilogram of body weight per day for 12 months). At 12 months, 39 of 65 patients (60%) in the rituximab group and 34 of 65 (52%) in the cyclosporine group achieved complete or partial remission. However, at 24 months, 39 patients (60%) in the rituximab group remained in remission, while the rate of remission in the cyclosporine group had dropped to 20% (13/65) due to a large number of relapses after the cyclosporine was tapered and stopped. Therefore, while rituximab was found to be non-inferior to cyclosporine in inducing remission at 12 months, it was statistically superior at 24 months in terms of maintaining long-term remission. In addition, among patients with anti-PLA2R-associated MN, the decline in

anti-PLA2R autoantibody titers was more rapid and more intense in the rituximab group than in the cyclosporine group. In terms of safety, there were no surprises and both agents were comparably safe and well tolerated.

There are several additional randomized controlled trials (RCT), comparing tacrolimus-rituximab to methylprednisolone-cyclophosphamide [30] and rituximab to steroids with cyclophosphamide NCT03018535, [77] in MN. The former shows the superiority of cyclophosphamide-based protocols in the adult while the latter is inconclusive. The best timing, dose and frequency of rituximab infusion in the adult remain to be determined.

Based on this data and on the extensive experience acquired in the use of anti-CD20 monoclonal antibodies, especially rituximab, in children with nephrotic syndrome [70], the use of rituximab appears reasonable as a first-line immunosuppressive agent, when available. Evidence of its efficacy in childhood forms of MN is very scarce and limited to case reports [60, 97]. In terms of dosing regimen and timing of infusions, especially in adolescents, using the same regimen as is advised for adults is appropriate. In children below the age of 12, the dosing most frequently used is 375 mg/m2/dose.

Other anti-CD20 agents, such as ofatumumab, ocrelizumab, and obinutuzumab, have also been employed in adults with MN. For ofatumumab, a single case report of a very young adult (18 years of age) shows effectiveness and safety [66], but this agent is no longer commercially available in most countries. Monitoring of long-term side effects, in particular relative to vaccine coverage and hypogammaglobulinemia, is important especially when anti-CD20 depleting agents are used in younger children, as long-term hypogammaglobulinemia has been reported in children with nephrotic syndrome [19]. Another B cell modulating agents, belimumab, which reduces naïve mature B cells but has less impact on B cell memory, appears to be potentially effective at 2 years of follow-up, both in reducing anti PLA2R antibody titers and in reducing proteinuria in a pilot study performed on adults with primary MN and nephrotic-range proteinuria [4].

Use of Antibody Titers to Guide Treatment in PLA2R-Associated Forms of MN A common goal of immunosuppressors is immunological remission defined by disappearance of PLA2R antibodies, a condition that usually precedes clinical remission. Conversely, persistence of high levels of anti-PLA2R Ab or their reappearance is usually associated with no remission and a relapse, respectively [41, 73]. This clinicobiological correlation seems to hold true for all antigens defined as yet, although prospective studies are needed.

Anticoagulation

Thrombotic events have been shown to be more frequent in the adult population with MN, irrespective of the degree of nephrosis. For this reason, KDIGO guidelines [93] suggest the use of prophylactic anticoagulation if serum albumin is below 2.5 g/dl. While this recommendation is probably not applicable to younger children, it may be prudent in severely nephrotic adolescents with MN.

Treatment of Specific Forms

HBV-MN can remit spontaneously in immunologically competent children, with seroconversion and clearance of HBeAg from the circulation [11]. In adults, studies have shown the effectiveness of interferon α, adenine arabinoside [54] and lamivudine [83], and some reports of the use of interferon α and lamivudine exist also for the pediatric population [10, 32]. In patients with a viral response to lamivudine without an immunological response as measured by anti-PLA2R antibodies, rituximab can induce remission of NS but continuation of lamivudine is mandatory [9].

In *neonatal MN induced by anti-NEP alloimmunization*, spontaneous remission is usually observed due to the short half-life of maternal antibodies. Neonates born after a second pregnancy are at major risk because of dramatically increased levels of anti-NEP antibodies on reexposure of the mother to placental NEP antigen. In these cases, which may present AKI, exchange transfusion may be necessary to remove the antibodies rapidly [28]. Monitoring of pregnancies in NEP deficient mothers relies on ELISA of anti-NEP antibodies with determination of IgG1 and IgG4 subclasses [91].

In *BSA-induced MN*, eliminating this protein by adopting a dairy-free diet could be beneficial. Circulating anti-BSA antibodies and BSA antigen should be monitored during follow-up because they correlate with disease activity [26].

Cross-References

- ▶ Autoantibodies and Kidney Diseases
- ▶ Lupus Nephritis
- ▶ Nephrotic Syndrome: Classification and Evaluation

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Part IV

Kidney Involvement in Systemic Diseases

Immunoglobulin A Nephropathy in Children

17

Norishige Yoshikawa and Rosanna Coppo

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Abstract

IgA nephropathy (IgAN), defined by dominant immunoglobulin A glomerular deposits, is the commonest glomerular disease in children presenting with microscopic hematuria. Its prevalence varies from 10% to 40% accounting for renal biopsy criteria and screening school programs. The pathogenetic events include mucosal production of galactose deficient IgA1, IgG autoimmune response, circulation and deposition of macromolecular IgA1 followed by complement activation, glomerular inflammation, and damage. The characteristic pathology feature is mesangial enlargement, with hypercellularity and matrix expansion. Renal pathology lesions are scored according to the Oxford Classification (mesangial hypercellularity M; endocapillary hypercellularity E; segmental glomerulosclerosis S; tubular atrophy/interstitial fibrosis T, crescents C) providing the MEST-C score. The most characteristic presentation is macroscopic hematuria associated with upper respiratory tract infections, sometimes with overt nephritic syndrome. Most children have microscopic hematuria with or without asymptomatic proteinuria, while a few have nephrotic onset. The outcome is generally favorable within the pediatric age, and up to 9% develop renal failure by 15 years. However, mild cases of IgAN in children might be missed and manifest irreversible damage only decades later. Hypertension, persistent heavy proteinuria, and reduced glomerular filtration rate at presentation and the MEST-C score provide prognostication indications. Not all children with IgAN need treatment as spontaneous remission may occur in mild cases. In children developing proteinuria angiotensin system, inhibitors are the first approach, while corticosteroids are an option for children with worsening proteinuria despite treatment or in cases with clinical and pathology features indicating active and potentially progressive IgAN.

Keywords

IgA nephropathy · Mesangial proliferation · IgA deposits · Galactose deficient IgA1 · Oxford classification

Introduction

IgA nephropathy (IgAN), first described by Berger and Hinglais in 1968, is defined by dominant or co-dominant IgA deposits in glomeruli [1] (Fig. 1). IgAN is the commonest glomerular disease not only in adults but also in children who undergo renal biopsy because of isolated microscopic hematuria or hematuria associated with non-nephrotic proteinuria [2].

The incidence of primary IgAN in children ranges from 5 and 140 per million/children/year

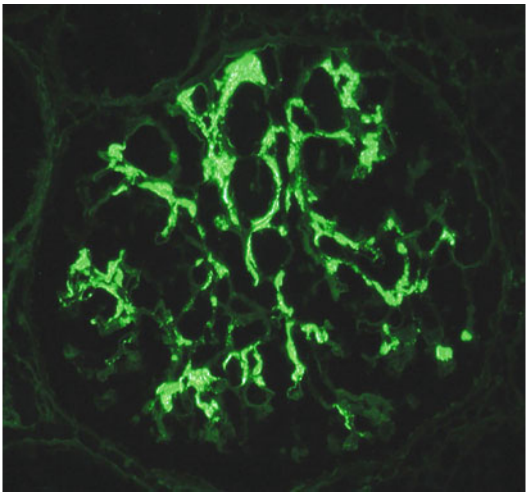

Fig. 1 Immunofluorescence micrograph showing mesangial IgA deposits in a patients with IgAN. Original magnification, ×400

similar to that in adults [3]. An international renal biopsy survey including 42,000 patients aged 0.1–100 years detected primary IgAN in 39.5% of renal biopsies in Asia, 22% in Europe, 12% in North America, and 6% in Latin America [4]. In children, IgAN represents 20–26% of renal biopsies in Europe [5], 17–20% in China, 14% in India, 10% in North America, 14% in South America, and 2.8% in Africa [6]. Conversely, IgAN is more frequent in Japan and Korea, where it represents 32% and 40% of renal biopsies in children [7, 8]. This variability may account for genetic and environmental factors, but most of all for school screening programs and variable criteria for performing renal biopsy. Renal biopsy follows the detection of urine anomalies by screening programs in some countries, while in others it is deserved to cases developing proteinuria [5]. In the large international cohort including patients of all ages, the different frequency of IgAN was related to the country of residence more than to the race/ethnic group [4], suggesting the role of environmental and lifestyle factors, as well as all local biopsy policies.

IgAN was initially considered a benign condition, but extended follow-up indicated a relevant progression over long term, involving 20–50% of all patients [9]. In children, the frequency of reaching renal failure within the pediatric age is limited by the short follow-up and increases when the observation is prolonged [10, 11].

Pathophysiology

Multiple mechanisms concur in the pathogenesis and progression of IgAN, including immunological, genetic, and environmental factors.

Genetic Factors

Familiar clusters of primary IgAN were reported in Kentucky and north Italy [12, 13]. Linkages of IgAN to 6q22–23, 4q26–31, 17q12–22 and 2q36 were observed, but no specific gene was identified. Several polymorphisms have been reported in association with IgAN development or progression, including genes encoding for Ig heavy-chain switch-region [14], core 1 beta1,3-galactosyltransferase (C1GalT1) [15], miR-146a [16], renin angiotensin system (RAS) [17], transforming growth factor-beta1 (TGFβ1) [18], platelet activating factor (PAF) acetylhydrolase [18]. Genome-wide association studies (GWAS) [19–21] have prioritized candidate genes implicated in the antigen processing and presentation pathway on chromosome 6p21 in the MHC region at three loci: (1) HLA-DRB1, -DQA1 and -DQB1, (2) HLA-DPA1, -DPB1 and -DPB2, and (3) TAP1, TAP2, immunoproteasome PSMB8, and PSMB9 genes [22, 23]. An increased expression of immunoproteasome, possibly regulated by these genes, was reported in patients with IgAN [24]. Other genes prioritized by GWAS are involved in the mucosal immunity pathway, including 22q12 (HORMAD2) locus, 8p23 α-defensin (DEFA) locus, 17p13 tumor necrosis factor (TNFSF13) locus involved in B cell development and class switching, 1p13 VAV3 locus (regulator of antigen presentation), 9q34 CARD9 locus (nuclear factor κB activation), 16p11 ITGAM and ITGAX (immune adhesion and phagocytosis), and the alternative complement pathway (chromosome 1q32 complement factor H (CFH) locus) [20, 23]. The association of CFHR3,1 deletion with reduced risk of IgAN [25] and altered serum levels of CFHR proteins detected in patients with IgAN [26] suggest a role for a dysregulated alternative complement pathway. Most loci associated with IgAN were also associated with immune-mediated inflammatory bowel diseases and mucosal response to intestinal pathogens, suggesting the hypothesis that the increased incidence of IgAN in some geographic areas might be consequent to a protective adaptation to mucosal invasion by intestinal pathogens [23]. A total of 15 IgAN susceptibility loci explained the overall risk of developing IgAN in 6.2% of European and 7.6% of Asiatic patients indicating the role of epigenetic factors.

Circulating IgA Molecules

The pathogenesis of IgAN is due to glomerular deposition of circulating IgA immune complexes (IgAIC) as indicated by electron-dense deposits in the mesangium coincident with IgA and C3 positivity. The role of macromolecular circulating IgA is supported also by the recurrence of IgAN in allografts and the disappearance of glomerular IgA in kidneys with subclinical IgAN transplanted by chance to non-IgAN patients [27].

IgA is the predominant class of immunoglobulin in mucosal secretions, where it consists mostly of dimers (polymeric IgA) [28]. In plasma, 90% of IgA is monomeric. Two isotypes IgA1 and IgA2 differ for a long insertion of amino acids at the hinge region between the CH1 and CH2 domains, which is present only in IgA1. IgA1 is the most frequent isoform in plasma, while both subclasses are found in secretions. IgA in renal deposits is mostly polymeric IgA1 [28]. IgA1 presents at threonine and serine residues in the hinge region insertion six short O-linked oligosaccharide chains (Fig. 2). A core N-acetyl galactosamine (GalNAc) occurs alone or is extended with β1,3-linked galactose (Gal) by C1GalT1 (which needs a specific chaperone, Cosmc) and covered with sialic acid in α2, 3 and/or α 2, 6 linkage [28]. In patients with IgAN, there is prevalence of IgA1 O-glycoforms consisting of GalNAc alone or sialylated [29–32]. This galactose-deficient IgA1 (Gd-IgA1) can form macromolecular self-aggregates and elicit IgG autoimmune response [33]. IgG antibodies recognize GalNAc-containing epitopes on Gd-IgA1, which cross-react with bacterial or viral cell-surface GalNAc residues. IgAIC constituted by Gd-IgA1 likely escapes clearance by hepatic receptors [34] and has a preferential renal deposition by virtue of enhanced lectinic reactivity with fibronectin, laminin, and collagen within the mesangial matrix [35].

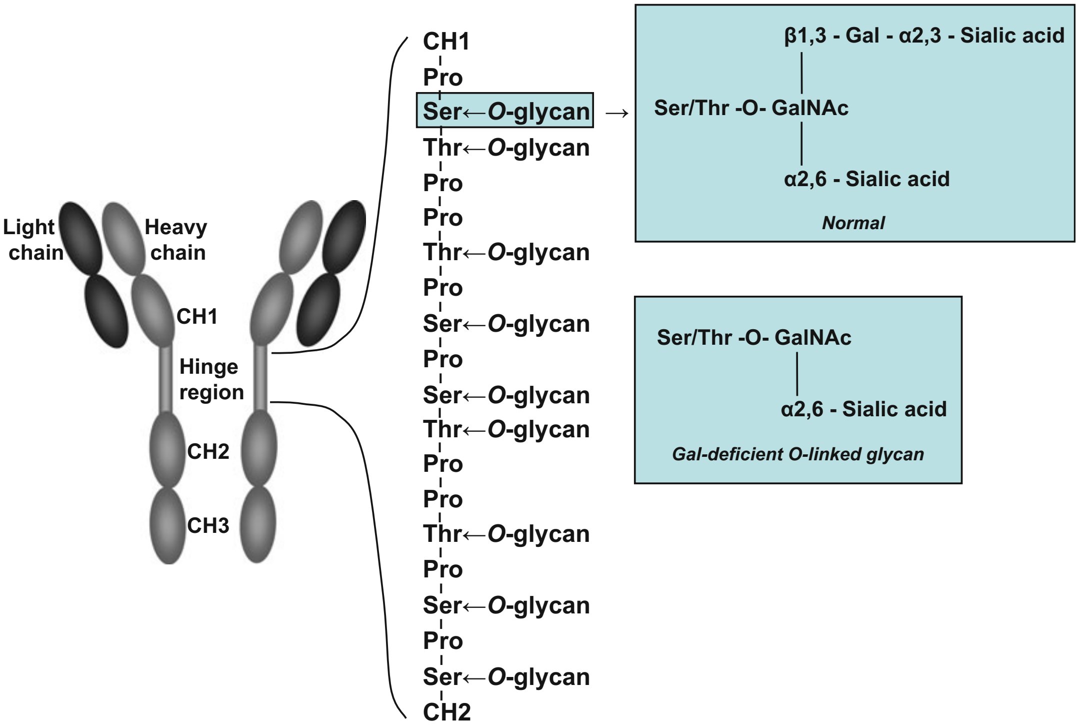
Light chain
Heavy chain
CH1
Hinge region
CH2
CH3
CH1
Pro
Ser←O-glycan
Thr←O-glycan
Pro
Pro
Thr←O-glycan
Pro
Ser←O-glycan
Pro
Ser←O-glycan
Thr←O-glycan
Pro
Pro
Thr←O-glycan
Pro
Ser←O-glycan
Pro
Ser←O-glycan
CH2
β1,3 - Gal - α2,3 - Sialic acid
Ser/Thr -O- GalNAc
α2,6 - Sialic acid
Normal
Ser/Thr -O- GalNAc
α2,6 - Sialic acid
Gal-deficient O-linked glycan

Fig. 2 IgA1 molecules with hinge region *O*-glycosylation sites. IgA1 molecules have two heavy chains with three constant region domains CH1–CH3 and a hinge region between CH1 and CH2. Each serine (Ser) and threonine (Thr) residue is a potential site for an *O*-glycan side chain. Although there are nine potential glycosylation sites, IgA1 contains up to six *O*-glycans per hinge region. *O*-glycosylation sites consist of N-acetylgalactosamine (GalNAc) *O*-linked to Ser or Thr residues of hinge region. The largest *O*-linked saccharide of IgA1 is a tetrasaccharide with GalNAc, galactose (Gal) and two sialic acid residues. Aberrant IgA1 molecules with Gal-deficient *O*-linked glycans can contain terminal GalNAc or sialylated GalNAc at one or more sites. Pro, proline

The multihit hypothesis of the pathogenesis of IgAN [36] (Fig. 3) considers as first step the aberrant synthesis Gd-IgA1, followed by a second hit of antiglycan antibody response, the formation of circulating IgA1IC (third hit), and their glomerular deposition, with complement activation, inflammation, and glomerular damage (fourth hit). In both children and adults, Gd-IgA1 are increased in circulation [37, 38] but also in healthy relatives, suggesting that elevated serum levels of Gd-IgA1 are heritable [39].

An additional role can be played by the interaction between human IgA1 and CD89, the soluble form of the IgA receptor [40]. The IgA1–CD89 complex interacts with transferrin 1 receptor (CD71) and transglutaminase 2 receptors (TGase2) expressed on mesangial cells leading to immune deposits and inflammation [41]. The deposition of Gd-IgA1IC and IgG/IgA1IC in the mesangial area, possibly amplified by the CD89 system, induces the release of inflammatory mediators and activates the complement pathways (Fig. 4). This results in mesangial proliferation, extracellular matrix expansion, oxidative stress, and inflammatory cells infiltration. Podocyte injury and apoptosis follows, mediated by mesangial messages and this induces glomerulosclerosis [42], while glomerular-tubular crosstalk triggers interstitial fibrosis [43]. In this process, activation of the RAS system plays a crucial role [44].

Complement Activation

Involvement of complement in IgAN is indicated by glomerular deposits of common pathway factors, for example, C3 and C5b-9, and deposits of specific pathway markers, properdin, CFH (alternative pathway) and mannose binding lectin (MBL), ficolins (lectin pathway) [45]. Complement activation induces glomerular cell injury and inflammation, leading to disease expression and progression [46]. Polymeric IgA and aggregated IgA activate the alternative and the lectin pathways of complement, while Gd-IgA1 selectively activates the alternative pathway [47]. C4d deposits in absence of C1q indicate lectin pathway activation. Although C4d and MBL deposits are not coincident, the origin of C4d production is still

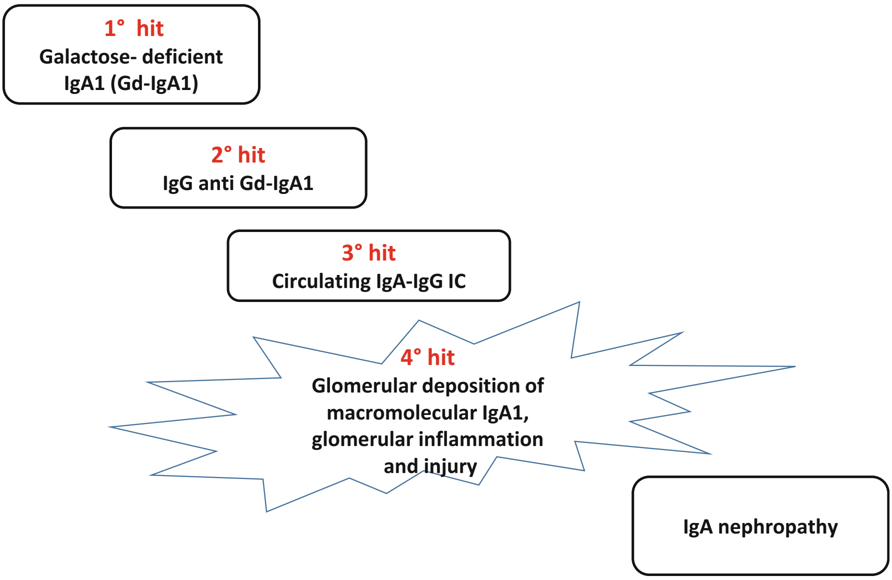
1° hit
Galactose- deficient IgA1 (Gd-IgA1)
2° hit
IgG anti Gd-IgA1
3° hit
Circulating IgA-IgG IC
4° hit
Glomerular deposition of macromolecular IgA1, glomerular inflammation and injury
IgA nephropathy

Fig. 3 Multihit hypothesis for the pathogenesis of IgA nephropathy: first hit, synthesis of Galactose-deficient IgA1 (Gd-IgA1); second hit, antiglycan IgG antibody response; third hit, formation of circulating IgA1-IgGIC; fourth hit, glomerular deposition of macromolecular IgA1, complement activation, inflammation and glomerular damage

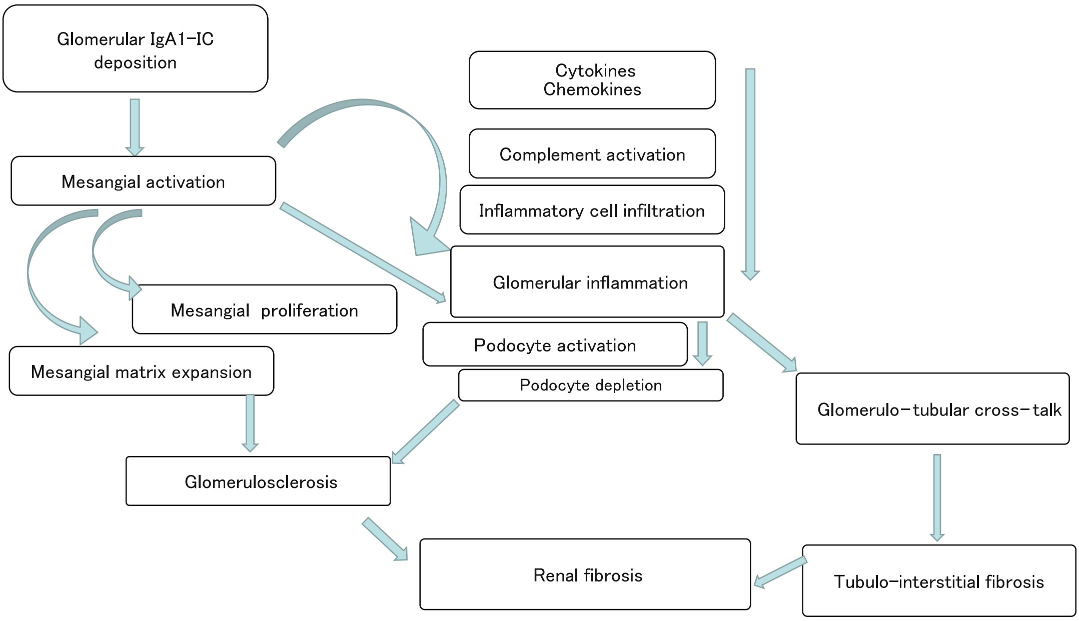
Glomerular IgA1-IC deposition
Mesangial activation
Mesangial proliferation
Mesangial matrix expansion
Glomerulosclerosis
Cytokines Chemokines
Complement activation
Inflammatory cell infiltration
Glomerular inflammation
Podocyte activation
Podocyte depletion
Glomerulo-tubular cross-talk
Tubulo-interstitial fibrosis
Renal fibrosis

Fig. 4 A simplified scheme of progression mechanism in IgA nephropathy

unclear [48]. The presence of C4d glomerular deposits is associated with progression of illness [49, 50]. These deposits co-localize with microangiopathic lesions in adults and children with IgAN and IgAN vasculitis [51]. Recent data have focused attention on subtle dysregulation of the alternative complement pathway in IgAN that represents a risk for progression [52, 53].

Innate Immunity, Mucosal Immunity

The feature of gross hematuria coincident with mucosal infections and detection of polymeric IgA in glomerular deposits has suggested mucosal immune response to environmental antigens as the first event in the pathogenesis of IgAN [54]. Mucosal IgA production is induced in the mucosal associated lymphoid tissue (MALT) by T-cell-independent and T-cell-dependent mechanisms [55]. The mucosal adsorption of intraluminal antigens triggers toll-like receptors (TLR), dendritic cell activation and synthesis of interleukins (IL-6, IL-10), TGF-β, B cell activating factor (BAFF), and APRIL (a proliferative inducing ligand). BAFF and APRIL, after binding to TACI (TNF receptor homolog transmembrane activator), actively promote the differentiation and proliferation of B cells with class switching from IgM to IgA [55]. Primed B cells migrate to the MALT effector area, the mucosal lamina propria, where they release dimeric IgA1 and IgA2. Renal deposits in IgAN mostly contain IgA1 subclass, which is a typical product of bone marrow plasma cells; hence, lymphocytes sensitized at mucosal level were supposed to migrate to bone marrow to differentiate into IgA1 producing plasma cells [56].

Several reports suggested that deposited IgA originate from mucosal challenge. The relationship between tonsils and IgAN was assumed after identification of disease, characterized by macroscopic hematuria shortly following pharyngotonsillitis with activation of the oral and Waldeyer pharyngeal ring (O-MALT). This hypothesis was supported by the cross-reactivity between antibodies eluted from the renal deposits of IgAN and tonsillar cells [57]. Tonsillar TLR9 was found to be more expressed in tonsils of patients with IgAN and it decreased after tonsillectomy [58]. Other studies failed to confirm the value of immune benefits of tonsillectomy in patients with IgAN [59].

The gut-kidney axis in IgAN [60] with dysregulated gut-associated lymphoid tissue (GALT) particularly represented by the Peyer patches was supposed after the observation of increased association of IgAN with celiac disease [61]. This hypothesis found a support in the GWAS study showing that most loci associated with IgAN are also associated with immune-mediated inflammatory bowel diseases, maintenance of the intestinal barrier, and response to gut pathogens [23]. Moreover, transgenic mice overexpressing BAFF develop hyper-IgA with IgAN conditioned by alimentary components and intestinal microbiota [62].

Intestinal microorganisms (microbiota) can modulate the GALT [63] and different microbiota patterns have been reported in patients with progressive IgAN [64]. In children and adults with IgAN, an increased intestinal permeability has been reported [65, 66] together with high levels of IgA anti alimentary antigens [38, 67]. Chronic enteritis was reported as second cause of mucosal infections in 35% of cases of IgAN from China [68], and this may favor increased release of bacterial lipopolysaccharide, which can methylate the chaperone gene Cosms, leading to production of Gd-IgA1 [69]. This hypothesis is supported also by increased expression of TLR4 (lipopolysaccharide specific ligand) in peripheral mononuclear cells, in children and adults with IgAN [70].

Apart from microbes, the GALT may also be triggered by abnormal response to alimentary antigens. IgAN was reproduced in mice after oral challenge with various alimentary components [71]. Mice fed a gluten-rich diet had significantly greater IgA mesangial deposits than animals fed a gluten-free diet since birth [72]. IgA anti-alimentary components and IgAIC were reduced after gluten-free diet in subjects with IgAN [73]. The role of gliadin was recently confirmed in IgA1/CD89 double transgenic mice developing spontaneous IgAN, in which a gluten-free diet for three generations prevented IgA deposits. The same study reported in patients with IgAN high levels of antigliadin IgA associated with increased proteinuria. These data suggest a role of GALT hyperresponse to intestinal alimentary components or microbes [74, 75].

Pathology

Immunohistologic Findings

The diagnostic immunopathological pattern of IgAN is the presence of IgA in the glomerular mesangium as the sole or predominant Ig. IgA deposits often extend just beyond the mesangiocapillary junctions into the adjacent capillary walls (Fig. 1). Lesser intense deposits of IgG and/or IgM may be seen, with the same staining pattern as IgA. In Yoshikawa series, mesangial IgA deposits were associated with IgG in 32% patients, IgM in 8%, and both IgG and IgM in 11% [76]. C3 deposits were observed in a similar distribution pattern in 64% cases. The early components of the classical complement pathway, C4 or C1q, are absent. Fibrin- or fibrinogen-related antigens are found in a diffuse mesangial distribution in 25–70% of patients and may be one of the injurious agents in the glomeruli [77]. Although, in most patients, IgA is present only in the mesangial regions, in ~10% of patients it is also seen in the peripheral capillary walls. Capillary wall deposits, seen on immunofluorescence or electron microscopy, are associated with severe clinical features and poor renal outcome [78–81].

Electron Microscopy

Electron microscopic abnormalities are mainly observed in the mesangium, which is enlarged by a combination of increased cellularity and matrix. Electron-dense deposits are seen in almost all patients (Fig. 5). They are granular masses situated immediately beneath the lamina densa in the perimesangial region and expanded mesangium. The size and extent of mesangial deposits vary from patient to patient; in some patients, they are large and produce localized protrusions. Peripheral glomerular capillary wall deposits are also found in the subendothelial and subepithelial regions. Subendothelial deposits occur most frequently in the capillary wall adjacent to the mesangium, although they are also observed in the peripheral part of the loop. Subepithelial deposits are unusual in adult patients,

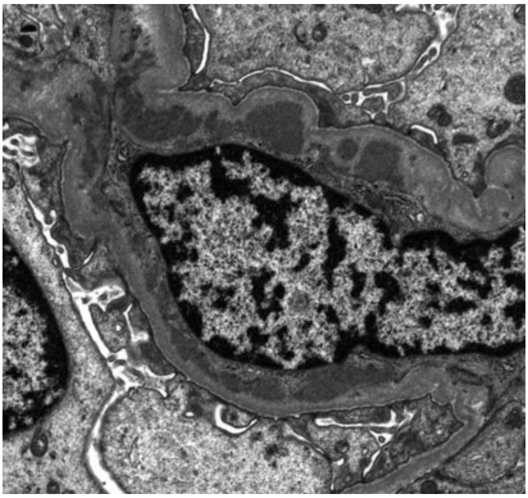

Fig. 5 Electron micrograph showing numerous electron-dense deposits in the mesangium in a patient with IgAN. Original magnification, ×5000

but are frequent in children with IgAN. They are small and flat and localized to a few capillary loops; humps typical of acute poststreptococcal glomerulonephritis are never seen. Lysis of the glomerular basement membrane is quite frequent in children [82]. In affected areas of the glomerular capillary, the lamina densa is thin and irregular, and the epithelial aspect of the glomerular basement membrane shows irregular segments of low electron density with an expanded, washed-out appearance. The epithelial foot processes are well preserved; diffuse foot process effacement may be seen in patients with nephrotic syndrome.

Light Microscopic Findings

Various glomerular changes are observed. The most characteristic abnormality is mesangial enlargement, caused by combination of hypercellularity and increase in matrix. Occasionally, small eosinophilic and PAS-positive fibrinoid mesangial deposits are also seen. Four types of mesangial change are identified in children with IgAN [83] (Fig. 6): (a) mesangial hypercellularity is more prominent than the increase in matrix, (b) the degrees of mesangial hypercellularity and matrix increase are similar, (c) the increase in matrix is more prominent than the mesangial cellularity, and (d) prevalent global glomerulosclerosis accompanied by reactive cell invasion. The first type of lesion is seen in biopsies in which the interval between onset of disease and biopsy is short. Serial pathologic observations reveal that prominent mesangial hypercellularity is almost exclusively seen in initial biopsies and decreases or disappears in follow-up biopsies. These observations suggest that predominant mesangial hypercellularity is characteristic of the early lesion of childhood IgAN and may disappear within a matter of months. An increase in mesangial cells is seldom striking in adult patients [84]. In contrast, biopsies with predominant matrix increase show a long interval between onset of disease and biopsy and a high percentage of glomerular sclerosis. Serial pathologic observations reveal that this type of change is usually seen in follow-up biopsies. An increase in the amount of mesangial matrix with duration of the disease has also been noted in adult patients [85]. These findings suggest that progression of IgAN leads to gradual decrease or resolution of mesangial hypercellularity and an increase of matrix associated with the development of sclerosis [86].

The severity of tubulointerstitial changes usually reflects the severity of glomerular damage. Vascular lesions, such as arterial or arteriolar sclerosis, are reported to be common in adults [87] but are unusual in children with IgAN [79]. This difference may be related to the age at biopsy and the duration of disease before biopsy.

Endocapillary hypercellularity is defined as an increased number of cells within glomerular capillaries, causing narrowing of the lumina. It may be present, in either focal segmental or diffuse distribution, and is typically associated with extension of deposits to peripheral loops. The hypercellularity might reflect proliferation, inflammatory cell infiltration, or endothelial cell swelling. Endocapillary lesions are more common in children and associated with active disease and high levels of proteinuria [88]. In IgAN, as in other proliferative glomerulonephritides, most of glomerular hypercellularity is due to leukocytes [89]. The presence of glomerular macrophages

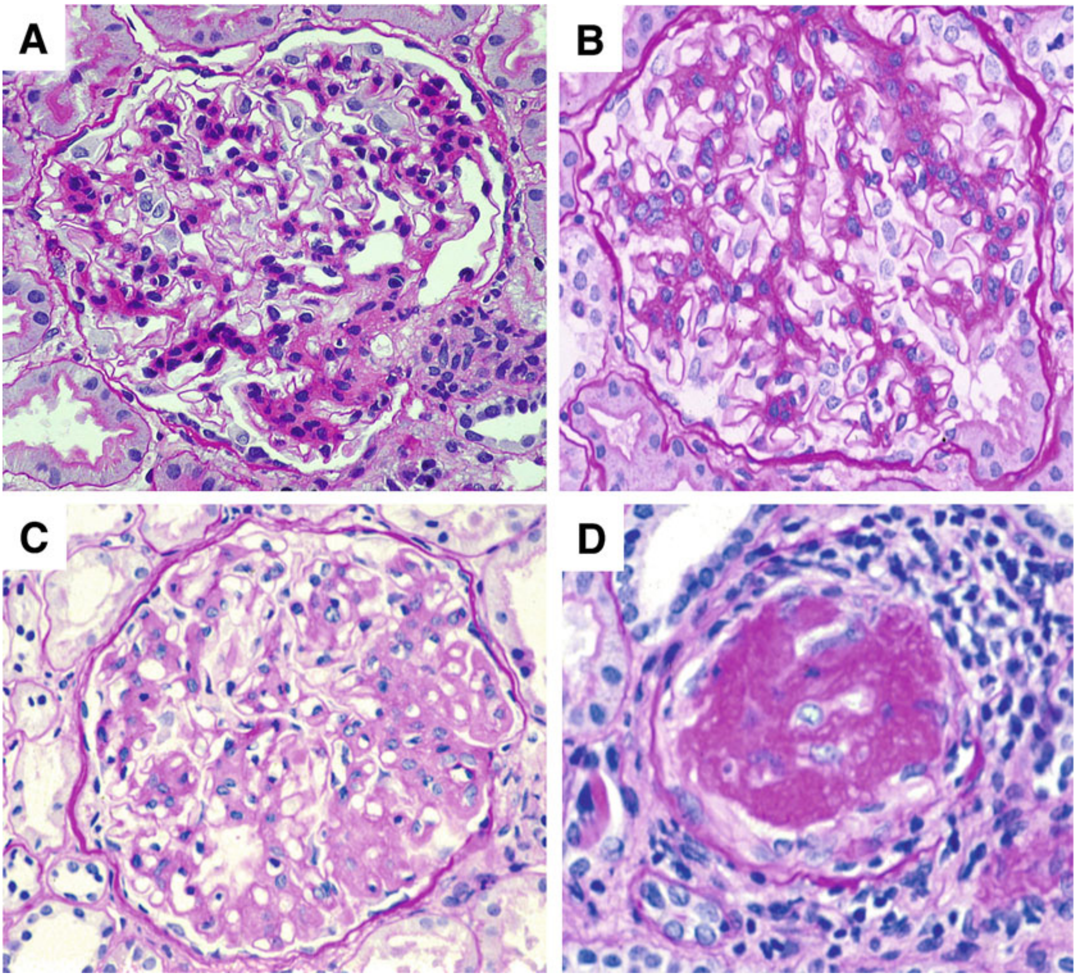

Fig. 6 Light micrograph showing mesangial proliferation in patients with IgAN. Four types of mesangial change are identified: (**a**) mesangial hypercellularity is more prominent than the increase in matrix. (**b**) The degrees of mesangial hypercellularity and matrix increase are similar. (**c**) The increase in matrix is more prominent than the mesangial cellularity. (**d**) Progression of IgAN is associated with the development of global glomerulosclerosis accompanied by reactive cell invasion. Original magnification, ×400

correlates with endocapillary hypercellularity and sclerosis [90].

Repeat Renal Biopsy

There have been only a few reports on the results of repeat renal biopsies [91, 92]. We previously reported our results in children with IgAN [93]. At the time of the second biopsy, 23 patients had showed clinical remission, defined as complete disappearance of proteinuria and hematuria with normal renal function, whereas 38 had persistent urinary abnormalities with normal renal function. There were no differences between the two groups with regard to the initial clinical and pathologic findings. The second biopsy of patients who were in clinical remission showed improvement of glomerular changes on light microscopy, a disappearance of or reduced mesangial IgA deposits, and lower amount of electron-dense deposits. Conversely, light microscopy showed a progression of histologic lesions and the persistence of both mesangial IgA deposits and electron-dense deposits in patients with persistent urinary abnormalities.

Shima et al. [94] have retrospectively screened and analyzed 124 consecutive children (age <18 years at first biopsy) with newly diagnosed severe IgAN showing diffuse mesangial proliferation, who received combination therapy (prednisolone + azathioprine or mizoribine + warfarin + dipyridamole, 90 patients) or prednisolone alone (34 patients) for 2 years and underwent repeat biopsies. After 2 years of treatment, 27 of the patients (21.8%) showed disappearance of glomerular IgA.

Differences Between Childhood and Adult Patients

Significant differences in the early glomerular lesions of IgAN between children and adults have been demonstrated [6, 95, 96]. Glomerular hypercellularity in mesangial area is prominent in children, and significantly greater than in adults. In contrast, glomerular matrix expansion and interstitial damage are more severe in adults compared to children. Glomerular hypercellularity correlated with proteinuria in children but not in adults, whereas glomerular matrix correlated with proteinuria and renal function in adults but not in children [95].

Oxford Classification

During past decades, many histologic classifications for IgAN were proposed, using semiquantitative or single-grade systems, and their utility in predicting renal outcomes. Although pathologic classifications provide valuable information for determining prognosis of IgAN, there is controversy as to whether these features add significant risk prediction to clinical features alone [97]. In 2009, the Oxford classification of IgAN reported the high predictive value of selected highly reproducible variables [98, 99] in patients with various age. The classification has obtained a considerable worldwide acceptance and was updated in 2016 [100]. The updated classification identified five key pathological features (mesangial hypercellularity, M; endocapillary hypercellularity, E; segmental glomerulosclerosis, S; tubular atrophy/interstitial fibrosis, T; and cellular/fibrocellular crescents, C) resulting in a MEST-C score that predicted renal outcome independently of clinical indicators at time of renal biopsy and during follow-up [98–100]. These findings are valid both for adults and children [100, 101].

Validation studies have demonstrated the clinical benefits and limitations and highlighted questions and difficulties of interpretation of biopsy specimens [102–109]. Findings from the VALIGA study, on 1147 patients with IgAN from 13 - European countries, have validated the Oxford classification across the whole clinical spectrum of the disease. Long-term follow-up of the cohort showed that the independent relationship between kidney biopsy findings and risk of progression towards kidney failure remains unchanged across age groups and decades after the renal biopsy [110].

Shima et al. [111] reported that a shorter time from onset to renal biopsy was associated with higher glomerular ratios of M, E, and crescents and lower ratios of S and G in 250 children with proteinuria >0.5 g/day/1.73 m^2 at biopsy, matching the inclusion criteria for IgAN of the original Oxford cohort. These findings indicate that acute lesions, such as M and E, reflect disease activity, with shorter times from onset to biopsy indicating severe disease activity. Crescents also reflect disease activity because crescents were acute lesions in their cohort. In contrast, chronic lesions such as S and G were absent early in course of disease, even in patients with severe acute lesions. Taken together, these findings suggest that each variable of the Oxford classification of IgAN may be influenced by the time from disease onset to renal biopsy and that differences in the significance of S and crescents between the original Oxford classification and other studies is due, at least in part, to differences in the timing of renal biopsy [111].

Finally, the most important clinical question is how we can make the Oxford classification more clinically relevant. Recently the International IgA Nephropathy Network used a large international collaboration of data sets with diverse ethnic representation to derive and externally validate a comprehensive risk prediction model in IgAN using readily available clinical and laboratory risk factors and the MEST-C score. The two adult prediction models were shown to be accurate and validated methods for predicting disease progression and patient risk stratification in adult multiethnic cohorts [112]. The updated pediatric prediction tool can predict the risk of 30% decline in eGFR or ESKD in children with IgAN [113].

Clinical Features

IgAN occurs at all ages but is most common during the second and third decades of life; it affects boys more often than girls, with the reported male to female ratio varying from less than 2:1 to 6:1 [114]. In a study of Japanese children [76], the mean age at presentation was 9.3 years in boys and l0.3 years in girls, and the male to female ratio was 3:2. The clinical presentation of IgAN varies. Some patients have asymptomatic microscopic hematuria with or without proteinuria. Other patients have recurrent episodes of macroscopic hematuria. Some patients present with acute nephritic syndrome and, more rarely, with acute renal failure.

Sixty-two percent of 258 Japanese children were found to have microscopic hematuria with or without asymptomatic proteinuria [76]. Twenty-six percent presented with macroscopic hematuria and 12% with an acute nephritic syndrome or nephrotic syndrome. Several studies from Europe and the United States reported that more than 80% of patients have episodes of macroscopic hematuria; recurrent gross hematuria is thus regarded as the hallmark of childhood IgAN [115–118]. However, it was the initial feature in only 26% of our series, presumably because of the school screening program that detected a high prevalence of asymptomatic urinary abnormalities rather than regional variation in the expression of IgAN. During the observation period, 60% patients had one or more episodes of macroscopic hematuria, whereas the other 40% were asymptomatic.

Macroscopic hematuria often occurs in association with upper respiratory tract infections; less frequently, it occurs in association with other infections involving the mucosal system (e.g., diarrhea and sinusitis). Episodes of macroscopic hematuria are sometimes associated with loin pain. The interval between the precipitating infection and the appearance of hematuria ranges from 1 to 2 days compared with 1 or 2 weeks in acute postinfectious glomerulonephritis. Many patients have recurrent episodes of macroscopic hematuria; each often associated hematuria is not with the same type of infection. The number of recurrences and the intervals between different episodes are variable. The incidence of macroscopic hematuria is lower in adults (Southwest Pediatric Nephrology Study Group) [118]. Emancipator et al. [119] summarized the previous reports of IgAN, in which most the patients were adults, and reported that 43% had macroscopic hematuria. However, in Japan, only 18–32% adult patients show macroscopic hematuria [120, 121]. The reason for the age-related differences is the incidence of macroscopic elucidated.

In asymptomatic patients, microscopic hematuria is almost always present and persistent. Proteinuria is common, and blood pressure and renal function at onset are normal. Patients with a nephritic or nephrotic onset have the most severe glomerular damage. Hypertension is infrequent and usually mild to moderate. Malignant hypertension is not a presenting feature in childhood. Nephrotic edema is reported in approximately 10% of patients. Acute renal failure is occasionally associated with episodes of macroscopic hematuria and is usually reversible. A number of investigators report a subset of patients with IgAN that is characterized by extensive crescents and a rapidly progressive course [121–123]. A review of published reports on crescentic IgAN revealed that 41% of patients with rapidly progressive glomerulonephritis were 16 years of age or younger [123].

Laboratory Investigations

Serum IgA levels are increased in 30–50% of adult patients but in only 8–16% children with IgAN [76]. For this reason, it is seldom of diagnostic significance. Serum complement component concentrations are usually normal, but the C3 level should be measured routinely if the patient has been referred for investigation after the first attack of hematuria to eliminate a diagnosis of postinfectious glomerulonephritis or membranoproliferative glomerulonephritis. Likewise, the antistreptococcal antibody titers should be determined after initial hematuria. These investigations are of little value when hematuria is known to have been present for more than 3 months. The serum creatinine should be measured routinely to estimate renal function and the estimated glomerular filtration rate should be determined. If present, proteinuria should be quantified, as proteinuria is associated with histologic lesions and a risk of progression. Plasma proteins should be measured routinely in the presence of heavy proteinuria.

Biomarkers

IgAN has been the focus of several studies aimed at identifying specific biomarkers. Serum galactose-deficient IgA1 level and glycan-specific autoantibody levels are prime candidates to become diagnostic biomarkers for IgAN because of their central role in the earliest stages of disease pathogenesis [124, 125]. However, although the serum level of galactose-deficient IgA1 is frequently elevated in patients with IgAN [37], the sensitivity and specificity of this laboratory finding are insufficient for the test to replace kidney biopsy as the diagnostic standard [2]. The serum level of glycan-specific IgG antibodies is correlated with the level of urinary protein excretion [126] and the risk of progression to end-stage renal disease or death [127]. This biomarker may prove useful for monitoring disease progression or the response to therapy [2].

Yanagawa et al. have reported that serum levels of galactose-deficient IgA1-specific antibodies are elevated in most IgAN patients, and their assessment, together with serum levels of galactose-deficient-IgA1, improves the specificity of the assays. These observations suggest that a panel of serum biomarkers may be helpful in differentiating IgAN from other glomerular diseases [128]; however, renal biopsy remains the only definitive diagnosis.

Urinary proteomic data are also candidates for diagnostic biomarkers because this approach can successfully differentiate patients with IgAN from healthy controls and from patients with other forms of renal diseases [2, 129]. Analyses of urinary samples by capillary electrophoresis with mass spectrometry have differentiated patients with IgAN from healthy controls and patients with minimal change disease or IgA immune complex nephritis due to chronic hepatitis C infection, even in association with nonpathologic proteinuria [2, 37, 126–132]. Furthermore, the urinary proteomic profile seems to predict the response to treatment with an angiotensin-converting enzyme inhibitor [133]. However, additional studies are needed to determine the potential and cost-effectiveness of urinary proteomic analysis in establishing the diagnosis of IgAN and making decisions about treatment [2].

Differential Diagnosis

The diagnosis of IgAN is based on the presence of IgA as the sole or predominant Ig in the glomerular mesangium. Because diffuse mesangial IgA deposits are observed in a variety of other disorders (Table 1), the diagnosis of IgAN can be made only by exclusion. IgA deposits are often incidental findings, and the pathogenesis and clinical significance are unclear.

Relationship Between IgAN and IgA Vasculitis (Henoch-Schoenlein) Nephritis

The relation between IgAN and Henoch-Schonlein nephritis (now IgA vasculitis nephritis, IgAVN) is complex. There is a close relationship between IgAN and IgAVN, with similar morphologic and immunopathologic features [134, 135]. The disorders are reported to coexist in different members of the same family, including a pair of monozygotic twins who developed the disorders

Table 1 Diseases associated with diffuse mesangial IgA deposits

Primary
IgA nephropathy
Secondary
Multisystem disease
IgA vasculitis nephritis (Henoch-Schonlein nephritis)
Systemic lupus erythematosus
Cystic fibrosis
Celiac disease
Crohn disease
Dermatitis herpetiformis
Ankylosing spondylitis
Neoplasms
Carcinoma of the lung and colon
Monoclonal IgA gammopathy
Mycosis fungoides
Non-Hodgkin lymphoma
Infections
Mycoplasma infections
Leprosy
Toxoplasmosis
Others
Chronic liver disease
Thrombocytopenia
Pulmonary hemosiderosis
Mixed cryoglobulinemia
Polycythemia
Scleritis

simultaneously after a well-documented adenovirus infection [136–138]. Moreover, the evolution of IgAN into IgAV in the same patient is described in both adults and children [139, 140]. It has been suggested that the two conditions are variants of the same process and that IgAN is IgAV without the rash [135, 138, 141]. Although there are similarities in their pathologic and immunologic features, the two conditions are clinically different, and the pathogenesis is not clear. Our study suggests that IgAVN is an acute disease, with glomerular lesions nonprogressive after the onset [142]. Therefore, in most patients, the prognosis is associated with the severity of glomerular change at the onset. In contrast, IgAN is a chronic, progressive glomerular lesion, which may eventually lead to progressive kidney disease, whatever the presentation. A few patients with IgAVN have recurrent episodes of macroscopic hematuria and progressive renal disease on repeat biopsies. Finally, IgAVN occurs mostly in young children and is rare in adults, whereas IgAN affects mainly older children and young adults. It is thus reasonable that these conditions be treated as different clinicopathologic entities until their pathogeneses is better understood.

Chronic Liver Disease

Glomerular IgA deposits may be observed in patients with various types of chronic liver diseases [143]. Mesangial proliferation and IgA deposits are the most common findings. Light, electron, and immunofluorescence microscopic features in patients with liver disease are similar to those in patients with primary IgAN. Most adult patients with chronic liver disease have clinically asymptomatic renal disease. Microscopic hematuria and mild proteinuria may be observed, but macroscopic hematuria, nephrotic syndrome, and impairment of renal function are rare. Glomerular lesions in children with chronic liver disease are very unusual. The pathogenetic mechanisms that contribute to mesangial IgA deposition in chronic liver diseases remain unknown. Significant elevations of the serum monomeric and polymeric IgA levels have been reported [144, 145]. Impaired hepatic clearance and increased synthesis of polymeric IgA, abnormal IgA metabolism, and portosystemic shunting of antigens and immune complexes are suggested as possible causes of mesangial IgA deposition in chronic liver disease [146].

Idiopathic Nephrotic Syndrome

A few patients with steroid-sensitive nephrotic syndrome show mesangial deposits of IgA on renal biopsy. They are classified by some as IgAN, whereas others consider that mesangial IgA in patients with minimal changes (i.e., without cellular proliferation) is coincidental. This probably applies to idiopathic nephrotic syndrome associated with mesangial IgA deposits occurring in

Asians and explains a favorable response to steroids, which is not the case in true IgAN [146].

Natural History and Prognosis

In adult series, the incidence of renal insufficiency varies from less than 10% to as high as 45% in long term follow-up in which 30–35% have been found to develop progressive renal insufficiency 20 years after disease initial discovery [114, 147, 148]. It can be estimated that 1–2% of adult patients will enter end-stage renal failure each year from time of diagnosis [149]. The long-term prognosis of 169 Japanese children with IgAN followed more than 10 years indicates that 9% of the patients had developed chronic renal failure by 15 years (Fig. 7).

Because of the variable rate of progression to chronic renal failure, there have been attempts to identify features present at the time of diagnosis that would predict the outcome. The following clinical findings are regarded as poor prognostic indicators in adult patients [114]: persistent hypertension, persistent heavy proteinuria, and reduced glomerular filtration rate at presentation [147, 148, 150–155]. In children, several studies have shown that the degree of proteinuria correlates with the severity of morphologic glomerular lesion [79, 117, 156], and heavy proteinuria at the time of biopsy predicts a poor outcome [80, 157]. In contrast, slight proteinuria or its absence at the time of biopsy predicts a favorable outcome [158]. Acute renal failure at onset is usually transient and associated with macroscopic hematuria and reversible tubular lesions. Males have been considered to have an unfavorable prognosis by some [147], but not all investigators [80, 159]. Schena et al. reported an increased risk of end-stage renal disease in familial IgAN [160].

Several pathologic features included in the MEST-C score are associated with a poor outcome as mentioned above, including diffuse mesangial proliferation; a high proportion of glomeruli showing sclerosis, crescents, or capsular adhesions; presence of moderate or severe tubulointerstitial changes; presence of subepithelial electron-dense deposit; and lysis of glomerular basement membrane by electron microscopy [80]. Patients with diffuse mesangial proliferation have been reported to have a significantly worse prognosis than those with focal proliferation or minimal lesions by light microscopy in adults [161, 162]. In many adult studies [87, 154, 159, 161], glomerular sclerosis and crescents have also been associated with poor renal outcome. Crescents were also associated with poor prognosis in children [117, 163]. Vascular lesions, such as arterial or arteriolar sclerosis, have been reported to play an important role in the

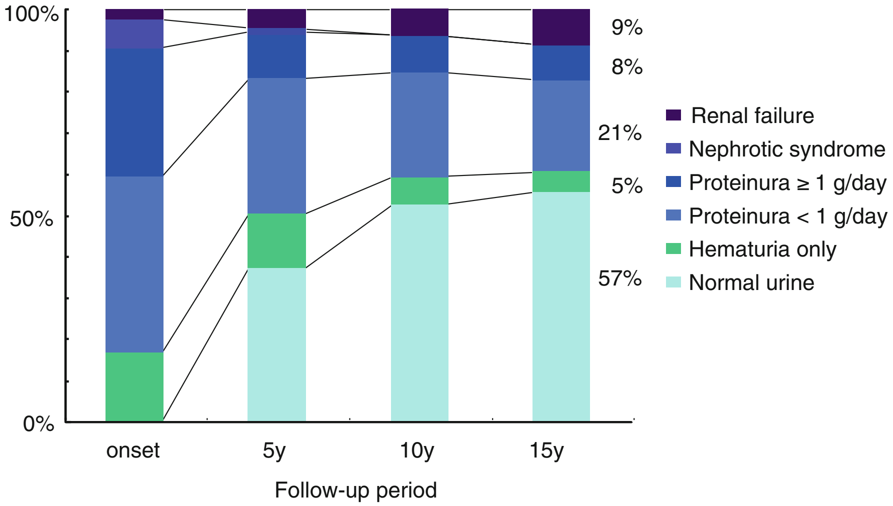

Fig. 7 Long-term prognosis of the 169 Japanese children with IgAN followed more than 10 years

progression of IgAN in adults. However, vascular changes are very unusual in children [83, 118, 122]. This difference may be related to the age at biopsy and the duration of disease before biopsy.

Our recent retrospective cohort study of 500 children with IgAN has showed an improved renal survival in Japanese children with IgAN, parallel to an increased use of angiotensin-converting enzyme inhibitors [164]. Among all patients, the actuarial renal survival was 96.4% at 10 years, 84.5% at 15 years, and 73.9% at 20 years. Diagnosed in 1976–1989, the renal survival was 94.0% at 10 years, 80.1% at 15 years, and 70.1% at 20 years. Diagnosed in 1990–2004, the renal survival was 98.8% at 10 years and 98.8% at 15 years ($P = 0.008$). In children with diffuse mesangial proliferation, both the 10- and 13-year renal survivals were 97.8% in 1990–2004, compared with 78.5% and 68.6%, respectively, in 1976–1989 ($P = 0.0003$). In the same study, prognostic factors for end-stage renal disease-free survival were analyzed [164]. The degree of mesangial proliferation and the year of initial renal biopsy were significant in both univariate and multivariate analysis. For children with IgAN, the most influential prognostic variable was the degree of mesangial proliferation. Proteinuria at diagnosis was significant in the univariate, but not in the multivariate analysis. Multivariate analysis showed that the initial renal biopsy year was a significant factor for renal survival independently of the degree of mesangial proliferation, proteinuria at diagnosis, and estimated creatinine clearance at diagnosis (hazard ratio = 0.08; 95% CI 0.004–0.43).

Some patients with IgAN achieve spontaneous remission even when not receiving medication. The retrospective study of 96 children with minor glomerular abnormalities or focal mesangial proliferation who did not receive medication showed spontaneous remission in 57 (59.4%) [165]. The cumulative spontaneous remission rates among these patients were 57.5% and 77.4% at 5 and 10 years from onset, respectively. The mean time from onset to remission was 5.9 ± 0.4 years. Of the 57 patients with spontaneous remissions, 10 (17.5%) also developed a recurrence of urinary abnormalities. The cumulative recurrence-free rates were 79.9% and 67.9% at 5 and 10 years, respectively, after remission. The spontaneous remission rate in childhood IgAN with minor glomerular abnormalities or focal mesangial proliferation was higher than expected [165]. These results suggest that physicians should consider the potential for spontaneous remission and refrain from aggressive treatment in IgAN patients with minor glomerular abnormalities or focal mesangial proliferation.

Long-Term Outcome After Renal Transplantation

IgAN may recur in approximately one-third of patients, more frequently in younger subjects and in those who had a rapid progression of the disease in the native kidney [27]. Overall graft survival rates at 5 and 10 years were 94% and 73% and the outcome was generally good in case of retransplantation [166]. Recurrence of IgAN did not affect the graft survival at over the first 10 years.

Management

Therapeutic Agents for Treatment

Renin-Angiotensin System Blockers

In children with IgAN, blood pressure control should be strict particularly when proteinuria is present. Blood pressure values must be targeted to the 50th percentile, as recommended by Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline [167]. The medications of choice are either angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) because they reduce blood pressure and proteinuria, and decrease mesangial cell activation through binding to specific receptors [44].

Coppo et al. performed a European multicenter RCT enrolling children and young patients (3–35 years old) with persistent proteinuria $>1 < 3.5$ g/day/1.73 m^2 [168] that were randomized to benazepril 0.2 mg/kg/day or placebo for 3 years. The primary outcome, identified by >30% decrease in eGFR and/or worsening of proteinuria to nephrotic range, was significantly better in the ACE-I group. A stable remission of proteinuria (<0.5 g/day/1.73 m^2) was observed in 56% of treatment group vs. 8% of placebo. On the basis of this trial and observations in adults [169], KDIGO suggested to treat children with IgAN and persistent proteinuria >0.5 g/1.73 m^2/day, with renin angiotensin system blockers. The effects of ACE-I in children with minimal proteinuria <0.5 g/1.73 m^2/day have not been investigated in randomized trials, but some benefits were suggested by a single arm collaborative Japanese observation over 2 years [170]. A recent RCT failed to show any benefit in adding ARB to ACE-I treatment in children with IgAN [171].

Glucocorticoids

The benefits of corticosteroids in IgAN reported in randomized trials in adults [172–174], led to the KDIGO suggestion that, if proteinuria persisted unchanged >1 g/1.73 m^2/day after 3–6 months of renin angiotensin axis inhibition, therapy with corticosteroids should be considered [167]. The KDIGO proposed a lower threshold of proteinuria to start corticosteroid treatment in children (>0.5 g/1.73 m^2/day). The statement was based on results obtained with 6 months regimens, using either three IV pulses of methylprednisolone (1 g) and oral prednisone (0.5 mg/kg on alternate days) [172], or oral prednisone 0.8–1 mg/kg/day for 2 months, with weaning over 6 months [173, 174]. Both protocols induced reduction in disease progression and proteinuria without serious side effects. A long-term legacy effect was observed after the pulse protocol [175]. No randomized controlled studies have been performed in children to validate these suggestions [176].

Some randomized trials in adults recently suggested a more cautious approach in using corticosteroids in patients with proteinuria and slow decline of renal functions. The STOP-IgAN trial [177] did not show a superior effect of immunosuppressive therapy (monotherapy with methylprednisolone pulses for 6 months in patients with eGFR >60 ml/min; combined treatment with oral prednisone and cyclophosphamide followed by azathioprine in patients with lower eGFR) versus supportive care for 3 years. The results at the end of the RCT were confirmed by a recent report after 10 years follow-up [178]. The original study as well as the follow-up report was underpowered to detect benefits of treatment because of the limited number of outcome events due to the slow progression of the illness in the cohort [179]. The Therapeutic Evaluation of Steroids in IgA Nephropathy Global (TESTING) study [180] randomized patients with proteinuria >1 g/day to either oral methylprednisolone or placebo. Recruitment was discontinued after 2 years because of excess serious adverse events (mostly infections, including two deaths) in 14% patients receiving corticosteroids versus 3% in the placebo group. While the preliminary results were consistent with potential renal benefit, early termination of the study did not allow any conclusions.

These recent studies in adults have influenced the most recent KDIGO guidelines for glomerulonephritis, which most of all address the severity of side effects and benefits of intensified supportive care [181]. The latter – not likely to be strictly adopted in children – consists of low-protein diet (0.8 g protein/kg/day), salt restriction, nicotine abstinence, treatment of metabolic syndrome, and blockade of the renin-angiotensin axis to achieve maximal blood pressure and proteinuria control. In normotensive children, the uptitration of ACE-I or ARB may be a challenge; similarly restriction of protein-intake might not be a suitable strategy.

In children, evidence-based reports on the effect of corticosteroids in treating IgAN are limited [176]. Uncontrolled observations or small studies in patients with varying baseline data and treated with different regimens provide conflicting results [182–184]. The best results were reported in severe cases with crescents successfully treated with pulse steroid therapy [184]. A placebo-controlled, double-blind randomized trial with prednisone (60 mg/m^2 every other day for 3 months, tapered to 30 mg/m^2 every other day for 12 months) or fish oil (4 g/day for 2 years) failed to show different outcomes [185]. However, the short follow-up, inequality of baseline proteinuria, and limited number of patients studied precluded valid conclusions.

Combined Corticosteroids and Immunosuppressive Medications

Some RCTs from the Japanese Pediatric IgAN Treatment Study Group supported the benefit of aggressive therapy in children with IgAN showing diffuse mesangial proliferation, which was considered per se a risk factor for progression. In these children, Yoshikawa's group performed the first pediatric RCT comparing a combination therapy (prednisolone, azathioprine, heparin-warfarin, and dipyridamole) versus heparin-warfarin, and dipyridamole for 2 years [186]. A decrease in proteinuria and mesangial deposition and a stabilization in number of sclerosed glomeruli was reported in the combination therapy group. The follow-up study revealed that 2 years of combination therapy improved the long-term outcome after 10 years [187] with better survival to the end point of eGFR<60 ml/min/1.73 m^2 (97% vs. 85% in anticoagulation arm). Another study enrolling patients with severe mesangial proliferation and comparing the effects of combination therapy vs. prednisolone alone showed additional benefits of the former [188]. However, side effects of prolonged anticoagulation and antiplatelet therapy have led to their discontinuation [189]. Azathioprine also needed frequent discontinuation, and mizoribine was used instead of azathioprine with improved tolerability [190]. In adults the addition of azathioprine to corticosteroids failed to prove further benefits and side effects were reported [191].

A recent retrospective study from Paris [192] examined children with severe clinical presentation and acute histologic features, who received therapy with either corticosteroids (sometimes in association with cyclophosphamide) and renin angiotensin axis blockers, or the latter alone. Although the baseline features were dissimilar for proteinuria and time from onset to treatment, a great benefit of corticosteroid/immunosuppressive therapy was reported, with improvement in eGFR and decrease in proteinuria after 6 months follow-up.

Mycophenolate Mofetil

Several small trials report conflicting results [193]. The KDIGO guideline does not recommend mycophenolate in IgAN, even though some benefits in Asian adult patients have been reported with a sparing effect on the total dose of corticosteroids [194]. A RCT that included children, young subjects, and adults examining the effects of corticosteroids, mycophenolate mofetil and fish oil was terminated after 6 months in view of failure to show any benefit [195].

Cyclophosphamide

This drug, in combination with corticosteroids, was tested in several uncontrolled case series. A small RCT [196] reported a protective effect of cyclophosphamide in progressive cases of adult IgAN. The STOP-IgAN study failed to show any benefits in patients with slightly reduced eGFR [177].

Rituximab

Because B cells may be involved in production of both Gd-IgA1 and anti-Gd-IgA1 antibodies, there is a rationale for considering anti-CD20 treatment in the condition. However, a small RCT of 34 patients that compared rituximab with supportive care failed to show an effect on eGFR, proteinuria, and Gd-IgA1 or anti-Gd-IgA1 antibody levels after 12 months [197].

Calcineurin Inhibitors

A recent meta-analysis reported limited effects of calcineurin inhibitors added to medium-low doses of prednisone and increased risk of adverse events [198].

Tonsillectomy

One of the first treatments considered for IgAN, particularly in children with recurrent gross hematuria, has been tonsillectomy. This procedure may theoretically eliminate pathogens which multiply in tonsils and remove macrophages and T cells, limiting Gd-IgA1 production. Several retrospective studies have analyzed the role of tonsillectomy in the treatment of IgAN mainly in adults [199]. Largely adopted in Japan, tonsillectomy in addition to steroid pulse was found in a RCT to provide a greater reduction in proteinuria in comparison to corticosteroids alone though the difference was marginal [200]. More recently a long-term follow-up reported a benefit on GFR decline in Japanese patients [199] and a meta-analysis concluded for some benefits in Japanese studies [201]. Tonsillectomy is indicated in children with IgAN when tonsils are a true infectious focus, or in case of recurrent tonsillitis (>3 episodes/year). In other cases the efficacy of the procedure has chiefly been reported from studies in Japan, and in association with other therapies.

Fish Oil/Omega 3 Fatty Acids

Data on efficacy of omega-3 fatty acids in children and adults with IgAN is limited, perhaps dose dependent, and not proven in a randomized study [185, 202]. KDIGO guidelines do not support this treatment in patients with IgAN.

Coagulation Modifying Agents

Warfarin, urokinase, and antiplatelet agents may add some benefits to corticosteroid treatment, but the side effects do not support their use in IgAN [187].

Future Therapies for IgAN

The enteric controlled-release formulation of the corticosteroid budesonide targets the Peyer patches at the ileocecal junction, which are pivotal for the GALT response [203]. Budesonide was tested in the placebo-controlled NEFIGAN phase 2b trial that showed a significant reduction of proteinuria and stabilization of eGFR over 9 months, without serious adverse events [204]. The drug is proposed to be tested in pediatric patients.

Combination of hydroxychloroquine and agents inhibiting the renin angiotensin axis showed significantly reduced proteinuria over 6 months in adults with IgAN [205].

New therapeutic approaches for IgAN include antagonists of the BAFF-TNF receptor family (BAFF, APRIL TACI). Trials with humanized monoclonal antibodies against these mediators are ongoing [38, 206]. Anticomplement drugs are under investigation for patients with IgAN, including anti-C5 (eculizumab), anti-C5a receptor inhibitors (CCX168), anti-C3 (compstatin), anti-factor D (lampalizumab), and MASP-2 inhibitors (OMS721) [38, 206].

Management of IgAN in Children

IgAN is no longer considered a benign disease and the diagnosis triggers the decision on need of treatment, choice of drug and duration [38]. No curative treatment is defined for IgAN: new studies target the pathogenetic events, including B cell and complement activation; however, results of these studies are not yet available [38, 206]. The identification of different risk levels at presentation, as recently offered to adult subjects with IgAN [112], should improve the selection of children to be treated and the choice of treatment [113]. A general approach to risk assessment, based on clinical and histological features, includes consideration of heavy proteinuria, mesangial proliferation, crescents, and glomerular sclerosis or interstitial lesions. Persistent proteinuria >0.5 g/day/1.73 m^2 is the most widely accepted indication for initiating treatment. However, lower levels of proteinuria (>0.3 g/day/1.73 m^2) might also be a risk factor over prolonged follow-up [207]. In children with acute onset, the variability in risk assessment due to timing of the biopsy [111] and the possibility of spontaneous improvement [165] limits the individual risk prediction at variance from adults, where a chronic and relentless progression is rather common [6]. Data from RCTs is scant and often inconclusive in children due to heterogeneity of risk factors and relatively short follow-up.

Hence, most data in children are derived from studies in adults, with the limitation of dealing with different expression of a same disease [6, 207]. A step forward to powered RCTs is to consider surrogate end-points in children as well as adults with IgAN, in order to shorten the observation time and increase the number of patients with better risk stratification. Proteinuria decrease is a widely accepted surrogate marker of reduced risk of progression [208]. Attention should be paid to conclusions from such surrogate markers, in absence of appropriate risk-matched control population.

Finally, there is a different attitude from clinicians in the selection of treatment for children and adults with IgAN: the choice in children with IgAN is often for a quick and powerful treatment, for fear of missing the active and reversible phase and not impair the subsequent sclerosis [6]. While we should refrain from treatments not based on evidence, the attempt to limit the progression of IgAN has to be individualized.

Selection of Patients

- Children with no risk for progression, for example, cases with isolated microscopic hematuria or gross hematuria completely resolved without any consistent persistent urinary abnormalities, should not receive treatment. Since the risk of progressive disease may change during the follow-up, regular re-evaluation is needed.
- A threshold value for considering treatment in children with persistent proteinuria, in addition to microscopic hematuria, is set at >0.5 g/g according to the KDIGO guidelines which recommend the use of ACE-I or ARB. Proteinuria values that exceed >0.3 g/day may be considered for this treatment.
- Children with clinical and pathology risk for progression should be offered 6 months corticosteroid treatment, either as IV pulses or as oral therapy. This group includes children with persistent proteinuria >0.5 g/g after 3–6 months RAS inhibition, or when values increase to >1 g/g in spite of RAS blockers or in cases with acute onset and worsening of renal function or active severe MEST-C scores. This choice remains opinion based, since no prediction formula integrating clinical and histological data suggesting specific treatment is presently available. The risk-benefit balance and steroid side-effects must be considered while making a decision regarding therapy.
- Children with nephrotic proteinuria associated with IgAN with minimal change disease features must be treated as those with minimal change disease alone.
- Children with acute kidney injury (AKI) in the context of macroscopic hematuria due to red blood cells and intra-tubular casts must follow the principles of supportive care for AKI.
- Cases with the rare variant of rapidly progressive glomerulonephritis and acutely decreased eGFR need to be treated as ANCA vasculitis, including the use of cyclophosphamide and steroid pulses. This protocol may be considered in patients with severe endocapillary hypercellularity or with crescent formation involving >30% of the glomeruli and a progressive course.
- The target of treatments is to reduce proteinuria <0.2 g/g. The follow-up must be continued in order to detect relapses that should be treated according to the new clinical features.

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Henoch Schönlein Purpura Nephropathy

18

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Abstract

IgA vasculitis, formerly known as Henoch-Schönlein purpura (HSP), is a clinical disorder defined as palpable purpura with one or more of arthritis/arthralgia, abdominal pain, renal involvement, or predominant IgA deposition. IgA vasculitis largely affects the pediatric population and generally has a favorable prognosis, with IgA vasculitis with nephritis being a feared outcome that contributes to poor prognosis. Renal involvement has a large range of symptoms, from microscopic hematuria to mixed nephritic-nephrotic syndrome with diminished renal function. The pathogenesis of IgA vasculitis with nephritis is similar to IgA nephropathy, suggesting a role of galactose-deficient IgA1 (GdIgA1) acting as an autoantigen, resulting in immune complex formation and deposition within the kidneys. Histology is indistinguishable between IgA vasculitis with nephritis and IgA nephropathy, with crescents and mesangial hypercellularity being hallmark features of IgA vasculitis with nephritis. Treatment recommendations are aimed at treatment of proteinuria when present, as well as attempting to reduce nephritis to prevent long-term damage by treatment with corticosteroids and immunosuppressive medications. Studies are currently ongoing to review clinical and histological factors that may suggest poor prognosis, with degree of proteinuria an important marker for outcome. Children with a history of IgA vasculitis with nephritis require long-term monitoring for adverse outcomes including hypertension, CKD, and ESKD.

Keywords

IgA Vasculitis · IgA Vasculitis with Nephritis · Henoch-Schönlein Purpura

Introduction

IgA vasculitis, formerly known as Henoch-Schönlein purpura (HSP), is a multisystem disorder that predominantly affects the skin, joints, gastrointestinal tract, and kidneys [1–5]. The diagnostic criteria for IgA vasculitis are presented in Table 1 [6]. The name and recognized elements of this condition have evolved. In 1801, Heberden reported a 5-year-old boy with abdominal pain, vomiting, melena, joint pain, petechial hemorrhages on his legs, and urine “tinged with blood.”

Table 1 Diagnostic criteria for IgA Vasculitis [6, 9]

Palpable purpura (mandatory criterion)
And one or more of the following:
Arthritis or arthralgia
Diffuse abdominal pain
Renal involvement (hematuria and/or proteinuria)
Leukocytoclastic vasculitis with predominant IgA deposit of proliferative glomerulonephritis with predominant IgA deposits

In 1837, Schönlein first described the condition in which arthralgia was associated with purpura. In 1868, Henoch described the gastrointestinal manifestations of purpura rheumatica, and 30 years later, he referred to nephritis as a complication. Other references to IgA vasculitis in early publications have used the terms of anaphylactoid purpura, purpura rhumatoide, and Schönlein-Henoch syndrome [7, 8]. The contemporary diagnostic term *IgA vasculitis* will be used in this chapter [5].

Epidemiology

IgA vasculitis (IgAV) occurs mainly in children, but adults can be affected [10]. While 90% of cases occur before 10 years of age, IgA vasculitis is rare among children younger than 2 years, peaks between ages 4 and 6 years, and has a declining incidence in adolescents and adults [11]. There is slight male predominance of 1.5:1 in children [12, 13]. The annual incidence is estimated at 3 to 27 pediatric cases per 100,000 children [11, 13–17] and 0.8 to 1.8 cases per 100,000 adults [10, 18–20]. The wide range in incidence in childhood appears to represent geographic variability, with incidence being reported as high as 55.9 children per 100,000 cases in Korea [21].

Though IgA vasculitis is the most common vasculitis in children, it generally has a good prognosis with the majority of children experiencing resolution of signs and symptoms. A study in the United States found hospitalization of children diagnosed with IgA vasculitis to be 2.4 per 100,000 pediatric population per year between 2006 and 2014 [22]. There is significant seasonal variation in disease presentation for children, with the peak incidence occurring during the fall and winter [11, 15, 16, 19, 23, 24]. There does not seem to be a similar seasonal variation for adult cases [10]. IgA vasculitis has a wide geographic distribution with appreciable variation. Clinical reports based on large patient numbers have originated mostly from Europe [14, 23, 25, 26], Finland [27, 28], and Asia [21, 29]. The condition appears to be less common in North America and Africa, but this may represent a reporting bias. Differences in disease risk have been postulated based on environment and ancestry, because IgA vasculitis is rare among African Americans in the southern United States [30, 31] and among persons from the Indian subcontinent [32]. In the West Midlands of England, white and Asian children had an incidence three to four times greater than black children [33]. No single infectious agent has been associated with IgA vasculitis, but observations suggest that 30–65% of IgA vasculitis cases are associated with viral or bacterial upper respiratory infections [34].

The proportion of IgA vasculitis patients with renal involvement ranges from 20% to 55% [35–37]. These variations may depend on the presence or absence of systematic urinalysis screening in IgA vasculitis cases, ambulatory or hospitalized sources of cases, and the location of care inclusive or exclusive of centers with nephrology expertise. Two studies in which routine urinalysis was performed for all patients admitted to the hospital for IgA vasculitis showed renal involvement in 41% and 61% of cases, respectively [38, 39].

Mechanisms of Disease

IgA vasculitis with nephritis (IgAVN) in children is usually an acute disorder with an abrupt onset [40]. The most accredited pathogenic hypothesis to date does not discriminate between IgA vasculitis and IgA nephropathy (IgAN), or the genetic background of these disorders. However, IgA vasculitis is distinguished by the degree of the systemic inflammatory response. A variety of stimuli ranging from infections by almost all types of pathogens, drugs, and allergens has been postulated to contribute to initiation of IgA vasculitis.

The multistep pathogenetic model for both IgA vasculitis with nephritis as well as IgA nephropathy begins with the synthesis of galactose-deficient IgA (Gd-IgA1). Gd-IaA1 behaves as an autoantigen and elicits production of autoantibodies of IgG or IgA class. IgG-Gd-IgA1 forms circulating immune complexes, which subsequently deposit in the mesangium. Upon immune complex deposition, mesangial cells react with multiple activation signals, inciting the release of cytokines and chemokines and amplifying the inflammatory response (Fig. 1) [41].

Role of Genetic Conditioning

The genetic predisposing background has been attributed to human leukocyte antigens (HLA) class I and class II genes [42]. In populations of European origin, HLA-class II DRB1 haplotype (HLA-DR1*0103 allele) and HLA class I B35 have been associated with susceptibility to the disease and to kidney involvement [43, 44].

Several polymorphisms or genetic variants within certain loci of non-HLA genes involved with autoimmune diseases or vasculitis and almost every gene encoding cytokines and chemokine involved with endothelial activation, adhesion, and inflammation have been explored [42, 45]. Among these, variants of genes encoding for interleukin 18 (IL-18), transforming growth factor beta1 (TGFB1), monocyte chemoattractant protein 1 (MCP1), selectin P (SELP), and Mediterranean fever – Pyrin (MEFV) were found to influence susceptibility to the disease. Variants in genes encoding for interleukin 1 beta (IL-1ß), interleukin 1 receptor antagonist (IL-1ra), TGFB1, interleukin 8 (IL-8), chemokine (C-C motif) ligand 5 (CCL5), and cytotoxic T-lymphocyte antigen 4 (CTLA4) influenced the occurrence and severity of renal damage. Variants in *MCP1*, intercellular adhesion molecule 1 (*ICAM1*), angiotensin, and *MEFV* seem to be involved in favoring gastrointestinal localization of disease manifestations. Endothelial genes involved in vascular reactivity, including nitric oxide synthase (NOS), renin angiotensin system (RAS), and the coagulation cascade, are possibly involved in the vasculitic process [46–50].

The abnormally galactosylated IgA (Gd-IgA) drove attention to the gene encoding β1-3 galactosyl transferase (GALT), and its chaperone core (1β3-Gal-T-specific molecular chaperone, COSMC) as determinants of glycosylation of IgA molecules [48, 51], especially since Gd-IgA are also observed in healthy relatives of the patients [52]. Most studies did not find significant difference in serum Gd-IgA between IgA vasculitis and IgA nephropathy. Aberrant glycosylation compared to the healthy

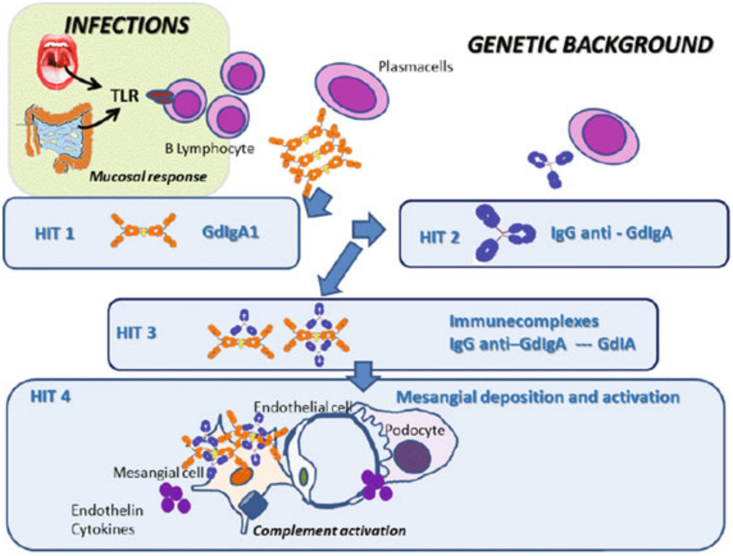

Fig. 1 Multistep pathogenetic model of IgA vasculitis with nephritis. TLR Toll-like receptor; Gd-IgA galactose-deficient IgA; IgG-Gd-IgA Immunoglobulin G galactose-deficient IgA antibody-antigen complex

population was confirmed. Antibodies at high titer against Gd-IgA are associated with most active cases of IgA vasculitis with nephritis in childhood [53].

Pathogenesis and Mechanisms of Disease Progression

IgA vasculitis with nephritis is characterized by inflammatory mechanisms in endothelial cells as well as mesangial cells, causing a strong vascular involvement both in the kidney and in other organs. The development of vasculitis is partially understood.

The endothelial cell is highly reactive and susceptible to cascades of complement, coagulation, and inflammation. One hypothesis is that IgA is generated from a cross reaction or molecular mimicry between the endothelial membrane molecules and the antigens expressed by the multiple pathogens colonizing the upper airways or the intestinal mucosae. These IgA molecules are directed against endothelial cells and induce vasculitis [54, 55]. Gd-IgA expose uncovered N-acetyl galactosamine (GalNAc) due to lack of the terminal Gal residue in the side glycidic chain of the IgA molecule. GalNac exposure is a common characteristic of the membrane of many pathogens and is therefore the appropriate self-reaction against Gd-IgA, being the second hit of the pathogenic chain [56]. IgA1 can bind to β2 glycoprotein, bridging IgA to phospholipids on the endothelial cell membrane [57], thus favoring amplification of thrombus formation and vasculitic damage. IgA1 binding to endothelial cells is enhanced by cytokines released during infections, such as tumor necrosis factor-α (TNF-α). Once bound to endothelial cells, IgA1 can bind to Fc receptor α expressed by circulating inflammatory cells, such as neutrophils favoring their adhesion to the endothelium and further attracting other inflammatory cells upon chemokines and complement fractions with chemotactic activity [58]. Upon IgA binding to endothelial cells via specific antigen recognition or via nonspecific binding via FcR α, a bridge is created attracting circulating cells to the vessel wall, amplifying the vasculitis through leukotriene and other endothelial products massively attracting neutrophil migration to the injured endothelium. Neutrophils release reactive oxygen species while bound IgA1 proceeds to activate the complement cascade resulting in endothelial cell lysis, necrosis, and formation of microthrombi.

Complement activation in IgA vasculitis with nephritis is recognized as one of the key pathogenic components. The classical, alternative, and lectin pathways can be triggered and activate the C3 and C5 convertases, resulting in the formation of the full membrane attack complex. In serum and on kidney biopsies, C3d, C4d, C5b-9, mannose-binding lectin (MBL), and mannose-associated serine protease 1 (MASP-1) staining have been observed [59]. C4d staining in the glomeruli and in the arterioles is frequently found in IgA vasculitis with nephritis and in active IgA nephropathy [60] and colocalized with microangiopathic lesions [61]. The coagulation system is more frequently engaged in IgA vasculitis with nephritis, compared to IgA nephropathy, with reduced serum factor XIII levels and increased expression of lipoprotein A (LPA), thrombomodulin, plasminogen activating factor (PAI-1), and S and C proteins [62].

The role of endothelial cells in the pathogenesis of rapidly progressive and acutely inflammatory forms of IgA nephropathy was valued in the Oxford classification that assigns a score to endothelial cell activation and damage. Endocapillary hypercellularity, E1, in the original cohort and in the other confirmative validation studies proved predictive of unfavorable outcome on univariate analysis in adults [63] and was an independent predictor of worse outcome with multivariable analysis.

The predictive value of endocapillary hypercellularity on outcome was stronger in IgA vasculitis with nephritis than in primary IgA nephropathy [64], though it is difficult to extrapolate the role of treatment in retrospective series. A large review of kidney biopsies in IgA vasculitis with nephritis is underway to determine morphological lesions of prognostic significance. Additional long-term follow-up studies are needed to validate the value of endocapillary hypercellularity or other lesions as prognostic indicators in children with IgA vasculitis [65].

Clinical Features

The extrarenal manifestations of IgA vasculitis are characteristic and can occur in any order across several days or weeks. The skin lesion typically begins with erythematous macules, some of which develop into slightly raised (palpable), urticarial papules. These early lesions may become purpuric (Fig. 2). The eruption is of symmetric distribution and predominantly affects the buttocks and the extensor surfaces of the lower legs and forearms but spares the trunk [12, 66, 67]. Purpura occasionally affects the ear lobes, nose, and external genitalia but in mild cases may be confined to the ankles. Among older children, purpura may be the sole cutaneous manifestation. As the primary lesions fade, new crops of purpura may recur 3 months after onset and occasionally much later [23, 25]. Cutaneous recurrence may be accompanied by the recurrence of abdominal, joint, and renal manifestations. A skin biopsy will demonstrate the presence of IgA deposits in small vessels, if needed to confirm the diagnosis [6, 12].

Joint symptoms are rarely the initial manifestation of IgA vasculitis, but ultimately occur in approximately 60 to 90% of children [12, 23, 25, 28, 69]. The spectrum of joint manifestations of IgA vasculitis consists of arthralgia, arthritis, and periarticular edema without overlying erythema, tenderness, or joint effusions. Joint involvement affects the lower extremities more commonly than the upper extremities [12, 28]. These symptoms are transient and leave no residual damage.

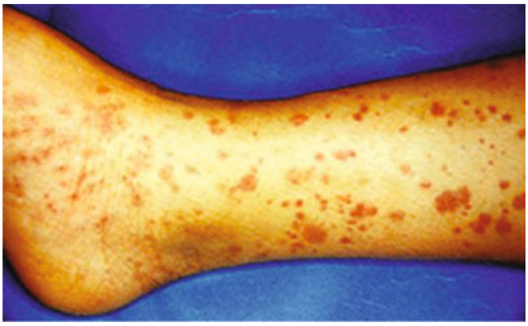

Fig. 2 Palpable purpura in a patient with IgA vasculitis [68]

Gastrointestinal manifestations are the presenting manifestation in 10–40% of cases and occur in 50–70% of children with IgA vasculitis [28, 70]. The incidence of gastrointestinal involvement increases the likelihood of renal involvement [23, 25, 66, 71]. The most common symptom is abdominal colic due to bowel angina [12], which often is severe and accompanied by vomiting. Melena occurs in one-half of cases [70]. As many as 22% of patients had occult blood in the stool, when GI manifestations were thought to have been evaded [28]. When the abdominal symptoms precede the other manifestations, laparotomy is sometimes considered necessary [25]. Intussusception due to severe purpura of the intestine is uncommon and mostly isolated to the small bowel [21, 72–74]. Protein-losing enteropathy has been reported [28, 75, 76] and may account for the occasional observation of hypoalbuminemic edema in the absence of heavy proteinuria. Rarely, abdominal colic may be attributed to ureteritis, resulting in obstruction of the ureter due to inflammation [77].Though uncommon, head imaging studies performed on patients diagnosed with IgA vasculitis and found to have altered mental status or focal neurologic lesions have identified intracranial hemorrhage, brain edema, and thrombosis of the superior sagittal sinus [78]. Vasculitic lesions have been found in many other organs and may explain rare manifestations such as pulmonary hemorrhage, acute hemorrhagic pancreatitis or parotitis, testicular hemorrhage, orchiditis, and myocardial or muscle involvement [28, 70, 77–80].

Renal manifestations occur at any time, with an incidence of ~25 to 50% [28, 35, 37, 69, 81, 82]. Renal manifestations may be minor, including microscopic hematuria or subnephrotic range proteinuria, or can be severe, including diminished eGFR, nephrotic syndrome, nephritic syndrome, or a mixed nephritic-nephrotic syndrome picture. The first urinary abnormality is usually noticed after other symptoms manifest. Among 80% of children that develop kidney involvement, the first abnormality is detected within 4 weeks of onset of IgA vasculitis. In most of the remainder, the urinary abnormality develops within the next 8 weeks [23].

A study of 238 children with IgA vasculitis showed that most patients (91.0%, 95% CI 85.0%–97.0%) developed abnormal urinalysis within the first 6 months [83]. The most common clinical manifestation was isolated microscopic hematuria or sub-nephrotic proteinuria in about 60% of the cases. Severe nephrotic proteinuria with kidney failure was identified in 30% cases. In a smaller proportion, ~20% cases, a rapidly progressive glomerulonephritis occurred, advancing to kidney failure in less than 10% of patients in the last 20 years. These outcomes were assessed during a period when widely diffuse immunosuppressive treatment was used with a goal to ameliorate the long-term risk for kidney failure [84–86].

Recurrences of renal manifestations are common and appear to be particularly so among patients with severe renal damage and older age at diagnosis. The relapses can occur at the time of an upper respiratory tract infection. There are no correlations between the severity of extrarenal and the renal symptoms. There seems to be no association between treatment with prednisone and prevention of recurrence of disease [28].

Several studies have been conducted to identify predictive factors for renal involvement in childhood IgA vasculitis [37, 87–92]. These studies show that age greater than 8 years at onset, persistent purpura, severe bowel angina, and relapse were identified as factors associated with the occurrence of renal involvement. This study also showed that patients with two or three risk factors developed nephritis in 63% and 87% of cases, respectively [93]. Minimal renal involvement may cause no more than transient microscopic hematuria, detectable only on routine urinalysis during the acute illness. The nephritis of IgA vasculitis may also present with macroscopic hematuria that usually lasts a few days. Macroscopic hematuria may persist for several weeks and microscopic hematuria may persist for many years. Macroscopic hematuria may be accompanied by transient heavy proteinuria. Persistence of heavy proteinuria of more than 1 g/m^2/day with microscopic hematuria is associated with significant histologic lesions, including crescents, endocapillary hypercellularity, and tubulointerstitial injury [94, 95].

Impairment of kidney function is uncommon and occurs with acute nephritic syndrome associated with combination of hypervolemia, oliguria, and hypertension [25]. Massive proteinuria can cause nephrotic syndrome (albumin <25 g/l) with pitting edema of the face and ankles and sometimes ascites. The most severe clinical presentation is mixed nephritic-nephrotic syndrome in which hematuria, hypertension, and impaired kidney function are combined with proteinuria and hypoalbuminemic edema [25]. Such a presentation is most often associated with crescentic glomerulonephritis or segmental glomerulosclerosis.

Pathology

The renal histopathology of IgA vasculitis is indistinguishable from that of IgA nephropathy; however, two basic types of glomerular lesions are the hallmark of IgA vasculitis with nephritis: mesangial hypercellularity and crescents.

Light Microscopy

Mesangial damage is the main feature and is characterized by variable hypercellularity, ranging from isolated mesangial hypercellularity to focal and segmental endocapillary hypercellularity with various extent of crescent formation. Figure 3 highlights endocapillary hypercellularity on light microscopy. Adhesions of the tuft to the Bowman capsule can be coincident with lesions of segmental hypercellularity, often with double contour appearance of glomerular basement membranes (GBM). A membranoproliferative pattern of injury may also rarely be present, due to mesangial cells and/or infiltrating macrophages/inflammatory mononuclear cells interposing themselves in the capillary loops between the endothelium and the GBM in response to subendothelial deposits. The ensuing matrix reaction, along with the interposed cells and deposits, gives

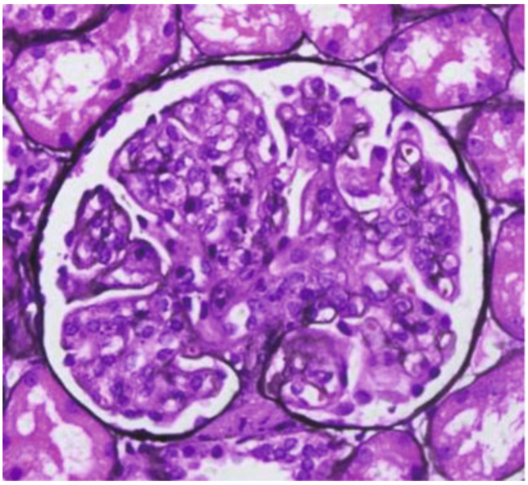

Fig. 3 Global endocapillary hypercellularity in a biopsy of IgA vasculitis (Jones silver stain, original magnification x 400)

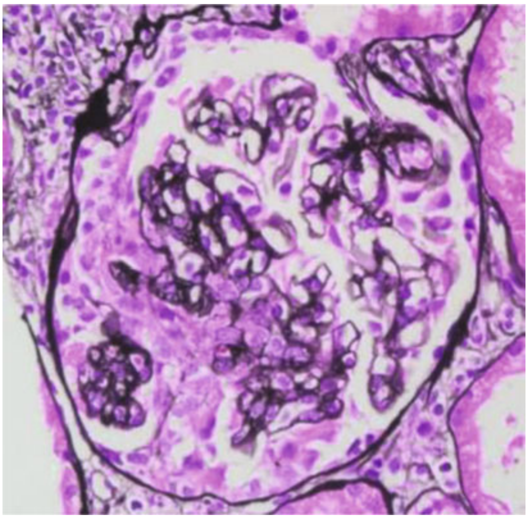

Fig. 4 Cellular crescent in IgA vasculitis in conjunction with segmental endocapillary hypercellularity, lower left (Jones silver stain, original magnification x 400)

rise to the double contour of the GBM observed on silver stain that stains only the matrix. Endocapillary hypercellularity occurs due to an increased number of cells within the glomerular capillaries causing narrowing of lumina. It may be focal or diffuse, and segmental or global, and is associated with extension of the deposits to the subendothelial location of peripheral loops. Hypercellularity may be attributed to inflammatory cell infiltration and endothelial cell swelling and/or proliferation. Endocapillary lesions are more common in children than adults and are associated with active disease. Crescents vary in size, from segmental to circumferential, evolve from cellular to fibrocellular to fibrous, and finally assume sclerotic aspects, with segmental or global scars. In mild cases, the crescents are small and limited to a small percentage of glomeruli, generally lower than 50% in association to endocapillary focal or diffuse hypercellularity [96–99]. Severe crescents, that involve more than 50% glomeruli, are seen in ~25% of the cases. Crescentic glomerulonephritis is most likely overrepresented, since patients with milder illness do not as often undergo biopsy. Figure 4 shows crescent formation with endocapillary hypercellularity.

Other lesions present in a minority of children are accumulation of fibrinoid material and necrosis of the tuft with inflammatory infiltration typical of vasculitis. Usually necrotic lesions are associated with diffuse crescents and acute inflammation. Tubular lesions are rarely observed in the acute phase, although acute tubular injury with flattening, vacuolization, desquamation, and focal loss of the brush-border microvilli may be seen. Tubular cylinders and blood casts are detected in 30% patients. When biopsy is delayed to over 6 months from the clinical onset, tubular atrophy with interstitial fibrosis and interstitial lymphocyte infiltration in areas of scarring are frequent, as seen in adults with IgA vasculitis. A small number of globally sclerosed glomeruli are often present. The membranoproliferative lesion described above may also more commonly be seen in the later stage biopsies.

Immunofluorescence

IgA vasculitis by immunofluorescence or immunohistochemistry is almost indistinguishable from IgA nephropathy. The diagnostic immunopathology feature is the presence of diffuse and usually global dominant or codominant IgA granular deposits in the mesangium. IgA deposits often may extend beyond the mesangiocapillary junctions into the adjacent capillary walls, and in some cases to peripheral capillary walls, most often associated with more hypercellularity (Fig. 5). Deposits of IgG and/or IgM with the same staining pattern of IgA are frequently present, and most often are of less intensity. IgA1 is the dominant subclass with polyclonal staining, often with slightly more lambda than kappa, in contrast to other immune complex disease where kappa is slightly more abundant than lambda. This is postulated to reflect the mucosal origin of the IgA deposits in IgA nephropathy and IgA vasculitis with nephritis, contrasting the bone marrow origin of the plasma cells giving rise to other immune complexes. The J chain is detectable, indicating that IgA in deposits is dimeric. The secretory component, synthesized by the epithelial cell to allow the trancytosis process across the epithelial cells, is absent. Extensive subendothelial deposits are associated with the most severe histologic forms with endocapillary hypercellularity and even crescents. C3 deposits are present in the majority, while the early fraction of complement cascade C4 and C1q is rarely present. C5–C9 and the alternative complement pathway component are instead always detectable. Fibrin or fibrinogen fractions are found with diffuse distribution in 25–70% patients, both in mesangial and parietal areas and coincident with mesangial hypercellularity, suggesting activation of the clotting cascade, particularly in cases with a high inflammatory status.

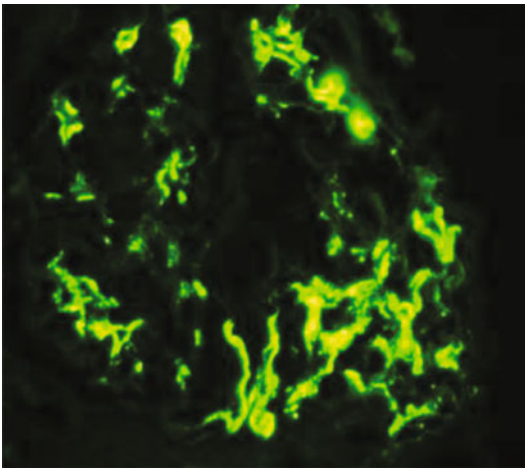

Fig. 5 IgA dominant staining in IgA vasculitis, with dominant mesangial staining with segmental capillary loop extension at lower right (anti-IgA immunofluorescence, original magnification x 400)

Electron Microscopy

The mesangium is variably expanded by a combination of increased cells, matrix, and deposits. Electron-dense deposits in the mesangium are present in almost all patients, but may be less conspicuous than expected from immunofluorescence findings (Fig. 6). The deposits have a granular aspect and are situated beneath the lamina densa, in the perimesangial region and in the expanded mesangium. The size is variable from very small to large and protruding. Peripheral glomerular capillary wall deposits are present mainly in the subendothelial areas and less frequently in the subepithelial areas in the capillaries adjacent to the mesangium, although also observed in the peripheral part of the loop. Frequent subepithelial or hump-type deposits should arouse suspicion for possible IgA dominant infection-related glomerulonephritis, particularly if there is a dominant glomerular neutrophil infiltrate with strong C3 staining. Clinical assessment and integration of all findings should allow distinction of these possibilities. The epithelial foot processes are well preserved; extensive foot process effacement may be seen in patients with nephrotic syndrome.

Comparison of IgA Vasculitis with Nephritis with IgA Nephropathy

IgA Vasculitis with nephritis may have a higher degree of likelihood for severe and acute pathology manifestations. Severe mesangial hypercellularity may progress to cellular interposition, inflammatory cells, and occasional mesangial cells interposing themselves into the capillary walls between the basement membrane and endothelial cytoplasm, typically in response to subendothelial deposits. The resulting basement membrane gives the capillary walls a “double contour” on silver staining, as detailed above. This membranoproliferative pattern of injury is

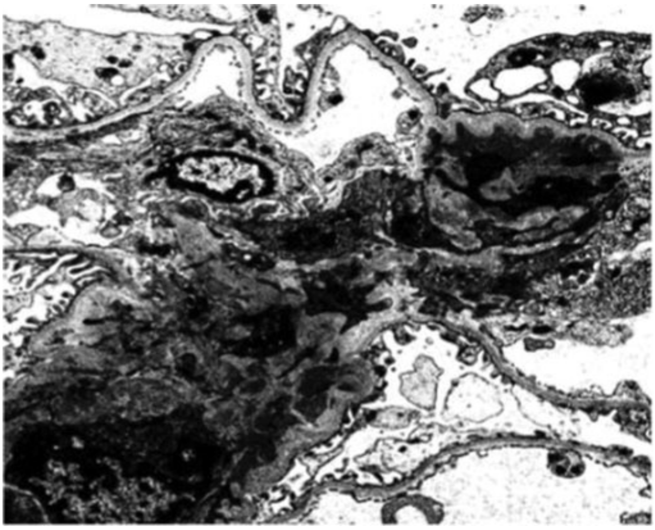

Fig. 6 Electron micrograph showing electron-dense deposits in the mesangium in a patient with IgA nephropathy [68]

only rarely diffuse. IgA vasculitis with nephritis compared to IgA nephropathy may have a greater degree of crescents. Endothelial activation typical of vasculitis leads to coagulation and complement cascade trigger, amplifying the endothelial damage and resulting in tuft necrosis. The released tissue factor, coagulation fractions, and fibrinolytic enzymes damage the GBM and infiltration of inflammatory cells. Release of these mediators, after GBM break, into the Bowman space stimulates the proliferation of parietal epithelial cells and influx of inflammatory cells, resulting in a crescent. Crescents reflect an extremely active, acute inflammatory process, often in parallel with clinical manifestations characterized by acute nephritic syndrome, rapidly progressive glomerulonephritis, hypertension, and gross hematuria.

Timing of Biopsy and Histology Findings

The presence of crescents is strongly dependent upon timing of kidney biopsy. Crescents may regress or evolve to sclerotic lesions when the kidney biopsy is postponed for months [100]. A recent large German collaborative study clearly demonstrated how timing of biopsy in children with IgA vasculitis with nephritis is crucial for the histological findings [97]. Cellular crescents were present in high percentage in early biopsies, within 15 days from clinical onset, while absent or partly evolved to fibrous crescents in late biopsies. The cases with a less abrupt clinical onset and milder renal symptoms may risk underestimation of the small percentage of crescents, depending on the timing of the biopsy and the adequacy of the biopsy specimen.

Histological Classifications and Their Significance

Three histological classifications have been developed. The inclusion of mesangial hypercellularity and crescent evaluation and quantification is common across the classifications and inclusion of acuity and chronicity elements varies. The generation of a classification measure inclusive of chronicity indices was in response to the low predictive value of long-term outcomes based exclusively on acute lesions in IgA vasculitis with nephritis [101].

ISKDC

This classification, developed by pathologists of the International Study of Kidney Disease in Children (ISKDC), is based on the degree of mesangial hypercellularity and proportion of glomerular crescents. ISKDC classes I–II include biopsies without crescents, while classes III, IV,

Table 2 ISKDC Classification [102] (Ref. [121])

I	Minimal glomerular abnormalities	
II	Pure mesangial proliferation	Focal or diffuse
III	Minor glomerular abnormalities or mesangial proliferation, with crescents or segmental lesions (sclerosis, adhesions, thrombosis, necrosis) in fewer than 50% of glomeruli	Focal or diffuse mesangial proliferation
IV	As III but with crescents or segmental lesions in 50–75% of glomeruli	Focal or diffuse mesangial proliferation
V	As III but with crescents or segmental lesions in more than 75% of glomeruli	Focal or diffuse mesangial proliferation
VI	Membranoproliferative- like lesion	Focal or diffuse mesangial proliferation

and V are defined by percent glomeruli with crescents categorized as <50%, 50–75%, or > 75%, respectively (Table 2) [102].

MEST

The Oxford MEST classification was designed for primary IgA nephropathy and considers mesangial hypercellularity, endothelial hypercellularity, segmental glomerulosclerosis, and tubular atrophy-interstitial fibrosis [103]. Crescents (cellular or fibrocellular; fibrous crescents are not scored) are scored as absent (C0), <25% (C1) or ≥25% C2 [104]. The MEST-C classification started to be used in children and adults with IgA vasculitis with nephritis to overcome the low predictive value of ISKDC. Validation initiatives require additional data and longitudinal assessments to assess the value of the MEST-C for children and adults with IgA vasculitis are underway. To date, distinct components of the MEST-C have been evaluated in small cohorts. M1 and S1 scores are reported to be more reliable in pediatric studies more frequently than in adults, while E1 and T1-2 scores have been useful in small cohorts of all ages [105]. Additionally, mesangial hypercellularity, segmental sclerosis, and tubular atrophy-interstitial fibrosis have been identified as prognostic for poor renal outcome. Segmental sclerosis on biopsy was associated with poor renal outcome (defined as 30% decrease in baseline eGFR, doubling of serum creatinine, or ESRD), with a hazard ratio 4.1 (CI 1.1, 15.0) compared with biopsies that did not have this finding [29]. Mesangial hypercellularity in greater than 50% of glomeruli (M1) and tubular atrophy-interstitial fibrosis of 25% or more of the biopsy (T1/T2) are prognostic of kidney failure (doubling of creatinine or ESKD) with hazard ratio of 4.0 (CI 1.2, 13.3) and 12.2 (CI 2.7, 55.3), respectively [105]. The predictive value of crescents (C0, C1, and C2) was confirmed in small adult cohorts, but not at multivariable analysis, which failed to show that crescents, the most impressive histologic feature of IgA vasculitis with nephritis, had a strong long-term predictive value for progression to kidney failure. Studies reviewing biopsies showing crescentic nephritis were found to have an odds ratio 3.9 (CI 2.4, 6.3) of developing poor renal outcomes, defined as hypertension, proteinuria >40 mg/m^2/h, increased serum creatinine, uremia, ESKD, dialysis, or transplantation [26, 29, 35, 105–108]. These studies are limited by their retrospective nature and varied treatments. Thus, C1 was associated with worse prognosis in IgA nephropathy only if no immunosuppression was given, while C2 lesions were associated with worse outcome regardless of immunosuppression [109–112].

Other Classifications

The low predictive value of histological lesions in IgA vasculitis with nephritis scored using different classifications leads to additional pathology grading efforts [113], mainly stemming from the original ISKDC or the MEST-C approaches. Among these, a recent work in a Finnish cohort of 53 children introduced a semi-quantitative description of lesions [114]. In this classification, a score was attributed to 14 parameters, crescents were distinguished as cellular or fibrous, and the different parameters were then combined into activity or chronicity indices. The performance of this classification awaits validation in larger independent cohorts. The low predictive value of crescents and the better performance of chronicity indices have been demonstrated in several other studies. In particular, sclerosis and interstitial fibrosis/tubular atrophy lesions over a 10-year follow-up are stronger negative predictors than crescents [65]. The absence of chronicity lesions, in particular if associated with low to normal excretion of urine protein, is considered predictors of better long-term kidney survival.

The predictive value of histology is low for IgA vasculitis with nephritis, in particular when classifications based on only acute lesions and crescents are used. This concept has been recently reminded in work that showed that low grade histology with mild smoldering proteinuria, receiving short term or no treatment may have worse long-term outcome than severe cases, aggressively treated to clinical and laboratory remission [115, 116].

Laboratory Investigations

IgA vasculitis is a diagnosis of exclusion, with no specific serum or urine findings. Of all pathogens linked to IgA vasculitis, group A β-hemolytic streptococcus is the most studied. Titers to anti-streptolysin O are raised in 20–50% of patients [117]. Further laboratory investigation is needed and has so far mainly been directed at assessment of the extent of renal involvement. If purpura is absent but is suggested by the patient's recent history, feces should be tested for occult blood, the finding of which may support a diagnosis of IgA vasculitis. If there is any doubt about the cause of purpura, a complete blood cell count and coagulation screen are performed. The level of clotting factor XIII, a fibrin stabilizing factor, is markedly low among children with IgA vasculitis. Although the serum IgA level is high in some cases of IgA vasculitis, measuring IgA levels for individual patients does not contribute to management.

In every case of IgA vasculitis, the urine is routinely tested for blood and protein at onset and at least weekly until systemic signs have resolved. A systematic review recommends that urine testing should be continued for 6 months, even if urinalysis is normal at presentation [118]. If urinary abnormalities are found, proteinuria is quantified, and serum levels of total protein and albumin are measured. Serum creatinine level is measured to estimate the eGFR.

Clinicopathologic Correlation

Renal biopsy is rarely needed for diagnosis, but it is used to assess the severity of glomerulonephritis [119]. Biopsy is required only in the care of patients with severe initial manifestations (nephritic, nephrotic, or decreased renal function) or with heavy proteinuria, for example, >1 g/day/m^2 that persists longer than 1 month. Biopsy for slight proteinuria, for example, <1 g/day/m^2, that persists longer than 3 months may be considered. The more severe the clinical presentation, the larger is the percentage of glomeruli affected by crescents and other hypercellular and/or sclerosing segmental lesions. The current clinicopathologic correlations in IgA vasculitis with nephritis aligned with contemporary pathology classification schemes are not well defined. However, a low activity and chronicity score portends excellent long term kidney survival, and high grade activity and chronicity might lead to a comparatively greater long-term risk for kidney failure [25, 86, 120–122].

Differential Diagnosis

IgA vasculitis is one of the small vessel vasculitides. The definition of the childhood vasculitides is based on the International Consensus Conference Nomenclature of Vasculitides (CHCC2012) [123, 124]. The distribution of the rash and the accompanying gastrointestinal and articular lesions are characteristic and, when combined with laboratory studies that exclude other small vessel vasculitis, the diagnosis is made [9, 119, 125]. Differential diagnoses of IgA vasculitis include other causes of purpura. Thrombocytopenic purpura and other vasculitides, such as hypersensitivity vasculitis, urticarial vasculitis, cryoglobulinemic vasculitis, cutaneous polyangiitis nodosa, and ANCA-associated vasculitis, must be differentiated. Difficulty arises in rare instances when the skin eruption consists entirely of urticarial lesions without purpura. Although it is not feasible to diagnose IgA vasculitis in the absence of cutaneous manifestations, there is anecdotal evidence that IgA vasculitis occurs without skin lesions, albeit rarely [126].

Among older children and adults, the combination of joint symptoms and purpura makes it necessary to consider systemic lupus erythematosus and microscopic polyangiitis as alternative diagnoses [127]. The light microscopic findings of glomeruli in patients with systemic lupus erythematosus and those with IgA vasculitis with nephritis are similar and may be indistinguishable. In systemic lupus erythematosus, however, glomerular IgA deposits, when present, are less prominent than IgG deposits, and C1q deposits are almost always present. Antinuclear antibodies usually are absent from patients with IgA vasculitis with nephritis. The differentiation between IgA vasculitis with nephritis and microscopic polyangiitis relies on antineutrophil cytoplasmic autoantibody titers (ANCA), for example, MPO and PR3, as well as the kidney biopsy. ANCA titers are typically elevated in microscopic polyangiitis and mesangial IgA, or any type of deposits, are absent or very sparse (pauciimmune necrotizing crescentic glomerulonephritis) [128].

Relationship Between IgA Nephropathy and IgA Vasculitis

The relation between IgA nephropathy and IgA vasculitis is complex, and there seems to be a close relationship [129]. The morphologic and immunopathologic features are similar in the two conditions [128, 129]. Serum levels of IgA are elevated in both IgA nephropathy and IgA vasculitis, and IgA-containing circulating immune complexes have been found in both conditions. The two disorders have been reported to coexist in different members of the same family, including a pair of monozygotic twins who developed the disorders simultaneously after a well-documented adenovirus infection [3, 31, 130, 131]. Moreover, the evolution of IgA vasculitis into IgA nephropathy in the same patient is described in both adults and children [132–134]. It has been suggested that the two conditions are variants of the same process and that IgA nephropathy is IgA vasculitis with nephritis without the rash [128, 131, 134]. Although there are similarities in their pathologic and immunologic features, the two conditions are clinically different. IgA vasculitis with nephritis tends to be an acute disease, with glomerular lesions that do not progress after disease onset [100, 120]. Therefore, in most patients, the prognosis is associated with the severity of glomerular change at the onset. In contrast, IgA nephropathy is a chronic, slowly progressive glomerular lesion, which may eventually lead to end stage kidney disease. A few patients with IgA vasculitis with nephritis have recurrent episodes of macroscopic hematuria and progressive kidney disease on repeat biopsies. Finally, IgA vasculitis with nephritis occurs mostly in young children and is rare in adulthood, whereas IgA nephropathy affects mainly older children and young adults [135]. It is therefore reasonable that IgA nephropathy and IgA vasculitis with nephritis be treated as different entities until their pathogeneses are better understood.

Treatment

Prevention of Nephritis

Steroids are effective in accelerating and favoring the resolution of arthritis and abdominal pain in IgA vasculitis; however, their effectiveness in the prevention of nephritis has been debated for decades. The fear of an unfavorable long-term renal outcome [99] prompted pediatricians to attempt preemptive treatment aimed at avoiding the occurrence of nephritis, mainly with oral steroids in short-term courses. The KDIGO 2012 guidelines included 5 RCTs with 789 children, where no protection on the development of persistent kidney involvement (microscopic hematuria, proteinuria, hypertension, reduced kidney function) at 6 and 12 months was obtained by 2–4 weeks of oral steroids. The KDIGO thus does not recommend the use of corticosteroids to prevent IgA vasculitis with nephritis (grade 1B) [136]. A recent placebo-controlled trial [137] also showed no evidence that systemic therapy with corticosteroids was associated with reduced occurrence of nephritis [138].

Treatment Options

The difficulty in determining therapy for IgA vasculitis with nephritis is small patient numbers with inability to evaluate treatment effectiveness and lack of data supporting one treatment regimen over another. Therapeutic choices are based not only on histology but also on the clinical scenario, though no specific therapeutic choice is supported by robust trials in children. The KDIGO 2012 guidelines produced three recommendations for the treatment of IgA vasculitis with nephritis in children: Treatment of persistent proteinuria with ACE-I/ARB; treatment of persistent proteinuria with GFR >50 mL/min/1.73 m^2 with corticosteroids after ACE-I/ARB is unsuccessful; and treatment of crescentic IgA vasculitis with nephritis with nephrotic syndrome and/or declining kidney function as crescentic IgA nephropathy with corticosteroids and cyclophosphamide [136].

The first recommendation suggests that children with persistent proteinuria >0.5–1 g/d/1.73 m^2 are treated with ACE-I or ARB for at least 6-months (evidence grade 2D), even if proteinuria increases. This recommendation was highly debated, particularly in Europe, where there is a long tradition of treatment with IV steroid pulses and immunosuppression in children aiming at the complete resolution of crescents and glomerular endocapillary infiltration [139]. The rationale is that of a strong anti-inflammatory treatment blunting the spontaneous evolution of crescents to chronic sclerotic lesions. Further randomized controlled studies are required in these patients to compare short-term prednisone therapy with placebo, to enable advice for treatment [140].

The second recommendation calls for treatment of persistent proteinuria with GFR >50 mL/min/1.73 m^2 with corticosteroids after failure of treatment with ACE-I or ARB for 6 months. The advantage of pulse steroids over oral therapy has not been addressed by specific clinical trials in IgA vasculitis with nephritis, where the tendency to use pulse steroids is widespread in aggressive illness aiming at a rapid anti-inflammatory effect. The indication to use pulse steroids in children is usually considered at a lower percentage than the 50% crescents threshold suggested in KDIGO 2012. In the 2019 update of KDIGO on glomerular disease, IgA vasculitis with nephritis is not specifically considered; however, the need for discussion and update of ACE-I and steroid use in IgA nephropathy is stated [141, 142]. This item has been recently addressed by the European consensus produced by the SHARE initiative (single hub and access point for pediatric rheumatology in Europe) on diagnosis and treatment of IgA vasculitis [143]. The initiative produced 26 recommendations, 21 of which based on expert opinion due to lack of published evidence. SHARE criteria of severity of IgA vasculitis with nephritis are mostly based on absence or presence of crescents in less or more than 50% of glomeruli, according to the ISKDC classification. A lower proteinuria threshold to start prednisone is not established. SHARE puts the prompt start of steroids as a recommendation, although without a high grade of evidence (level 4, strength D). According to SHARE, mild cases of IgA vasculitis with nephritis with normal renal function and mild to moderate proteinuria deserve first line treatment with oral steroids. Pulses in association to immunosuppressive medications (azathioprine, mycophenolate mofetil, cyclosporine) are suggested as second line for more severe or resistant cases. Moderate IgA vasculitis with nephritis defined by GFR reduction or persistent proteinuria in the presence of $<50\%$ crescents requires a more aggressive treatment with oral or pulse corticosteroids as first line. The acute explosive onset of IgA vasculitis with nephritis in a high percentage of cases manifests clinically with rapidly deteriorating kidney function sustained by crescents in a high percentage of glomeruli. These cases can be assimilated to rapidly progressive IgA nephropathy or to vasculitis, and the treatments suggested by KDIGO recommendation follow the clinical practice for vasculitis [136].

The third recommendation advised treatment of crescentic IgA vasculitis with nephritis with nephrotic syndrome and/or declining kidney function treated as crescentic IgA nephropathy with steroids and cyclophosphamide, despite little evidence to support cyclophosphamide use in IgA vasculitis with nephritis. Cyclophosphamide was found to be useful to induce remission of proteinuria in a study in children, but the results were not conclusive when considering a protective effect on renal function or progression to renal failure [144]. In this RCT, no benefit of cyclophosphamide over placebo was reported but this trial suffered from the bias of late enrollment, several months after onset, as well as small sample size [144]. In a study of 54 adults with IgA vasculitis with nephritis, no additional benefit was demonstrated by adding cyclophosphamide to prednisone baseline therapy in 25 randomized patients versus 29 patients receiving steroids alone [145]. KDIGO 2012 guidelines for IgA vasculitis with nephritis included immunosuppressive drugs with a low evidence of efficacy (grade 2D) even in case of deteriorating renal function [136] and a systematic review was not conclusive on indications in 2015 [140].

Immunosuppressive Agents

Although not supported by controlled trials and guidelines, the use of immunosuppressive medications is widespread both in adults and in children with IgA vasculitis with nephritis. Many immunologically active drugs have been studied but most are small, uncontrolled series or case reports. Recently, the CureGN study, including 285 children (112 IgA vasculitis with nephritis and 173 IgA nephropathy) and 382 adults (49 IgA vasculitis with nephritis and 333 IgA nephropathy), reported use of immunosuppressive drugs at diagnosis in both children and adults: 16.3% of adults versus 8% of children [135]. The Cure GN study reports that in the last years, children were more frequently treated with mycophenolate mofetil (MMF) (20.5% in children vs. 10.2% in adults). Azathioprine was still used both in children and in adults (8.9% vs. 6.1%). In this cohort, adults had a more severe IgA vasculitis with nephritis, with renal failure and the need for aggressive treatment including steroids in the majority of the cases (85.7% in adults and 73.2% in children).

In clinical practice, however, particularly in rapidly progressive cases or with steroid resistant nephrotic syndrome, immunosuppression is generally used. The most frequent choice is between cyclosporine [146, 147], tacrolimus [148], and MMF. MMF is gaining favor in recent years due to limited side effects, satisfactory safety profile, and the clinical confidence derived from other types of nephritis, in both adults [149, 150] and children [151–154]. In a recent study, MMF was introduced in 18 children as early second-line therapy, within 3 months after the diagnosis of IgA vasculitis and 1 month after nephritis onset, after steroid pulses with incomplete response (33.8 ± 47.6 days after the diagnosis of IgA vasculitis with nephritis and 21.9 ± 17.5 days after initiation of pulsed methylprednisolone) [152]. The initial dose of MMF was 800–1200 mg/m^2/day, and remission of proteinuria was obtained within 3 months without significant side effects.

Cyclosporine A was compared to methylprednisone pulses in a study on 65 children with IgA vasculitis with nephritis, with similar results on long-term follow-up [146, 147, 155]. The use of rituximab (RTX) is also promising in children, extrapolated from experience in nephrotic syndrome and in other types of vasculitis. After initial case reports in adults [156–161] and children [162], small cohorts were published in recent years. RTX was effective in inducing remission of proteinuria in cases resistant to attempts with multiple drugs and in patients with steroid dependence without significant side effects [163]. Remission of proteinuria, stabilization of renal function, and absence of relapses were observed in 90% of patients who were treated with RTX. The dosage regimens were variable, from 2 doses of 750–1000 mg/m^2 administered 2 weeks apart to 4 weekly pulses of 375 mg/m^2. A review of findings in 22 adults, who were either steroid resistant or had contraindications to steroid therapy, reported some benefits on proteinuria reduction and prevention of GFR decline. To date, the published reports in children and adults are based on uncontrolled data with limited value as guidelines [164]. The alternative anti-CD20 monoclonal antibody, ofatumumab, was described as equivalently effective in one patient with IgA nephropathy [165]. There is anecdotal experience with the use of plasma exchange [166] and eculizumab [167, 168].

Long-Term Course

IgA vasculitis is typically self-limited with overall good prognosis and high probability of full renal function recovery within 6 months [100, 169]. However, relapse of disease is common among children (recurrence rate, 2.7%–66.2%) [170–172]. The long-term morbidity and mortality of IgA vasculitis are almost exclusively attributable to renal disease. Many observational studies have been conducted to elucidate the demographic and clinical characteristics associated with long-term outcome [23, 29, 94, 106, 122, 173, 174]. The difficulty in identifying clinical features or histologic findings associated with poor renal outcomes is the potential prolonged period of time between disease presentation and development of hypertension, chronic kidney disease, and end stage kidney disease.

Hypertension

Hypertension at presentation of disease has not been identified as a prognostic marker for poor renal outcomes. However, patients with a diagnosis of IgA vasculitis with nephritis are at a greater risk of developing hypertension compared with the general population. A case-control study evaluated the UK primary care database on 10,405 patients with childhood-onset and 2,828 patients with adult-onset IgA vasculitis, with age- and sex-matched controls. In this study, a 44% increase in emergent hypertension (cases 1.34% vs. controls 0.93%) in childhood onset IgA vasculitis was reported [173].

Complications During Pregnancy

A history of IgA vasculitis seems to increase the risk of complications during pregnancy. In a retrospective study of 44 women with history of IgA vasculitis, 36% of 44 pregnancies were complicated by hypertension and/or persistent proteinuria [86]. Another retrospective study from Australia reported 247 pregnancies in 121 women with a previous diagnosis of IgA vasculitis. They found that 10.9% (27/247 pregnancies) of women with a history of IgA vasculitis had gestational hypertension compared with 1.7% (10/556 pregnancies) in matched controls, with an odds ratio of 4.7 (95% CI 2.3, 9.8) [174].

Chronic Kidney Disease

Based upon published literature, 13–30% of patients with IgA vasculitis with nephritis develop CKD; the risk factors are summarized in Table 3 [26, 106, 114, 175, 176]. The UK primary care database study reported a 94% increased incidence of CKD in the population with a history of IgA vasculitis compared with controls (case 0.31% vs. controls 0.16%) [173]. CKD outcomes have largely been attributed to the presence and severity of nephritis in the acute phase. In a long-term study with mean follow-up of 23 years, 22% (17 of 78 children) had diminished renal function [86]. A more recent study found similar proportions, with 30% (47 of 159 patients) having poor renal outcomes at a mean follow-up of 37 months [107].

The presence of decreased eGFR and nephritic or nephrotic syndrome are risk factors for developing CKD [29, 106–108, 122, 177]. A meta-analysis found that nephrotic syndrome and mixed nephritic-nephrotic syndrome at presentation were associated with increased likelihood of poor renal outcome by 1.7 times and 4.5 times, respectively, compared with no renal involvement or minor urinary abnormalities, such as hematuria and/or non-nephrotic proteinuria. Poor renal outcome in this study was defined as hypertension, proteinuria >40 $mg/m^2/h$, increased serum creatinine, uremia, end stage

Table 3 Risk factors associated with prognosis of kidney health in IgA vasculitis

Kidney health indicator	Risk estimates (CI)	N	Reference
Risk factors for chronic kidney disease			
Proteinuria at onset	RR 1.09 (1.01, 1.18)	219	[178]
Persistent proteinuria	RR 1.44 (1.27, 1.64)	219	[178]
Impaired eGFR at onset	RR 1.97 (1.02, 3.78)	219	[178]
Interstitial fibrosis; tubular atrophy	$P = 0.009$	159	[107]
Risk factors for ESKD			
Persistent proteinuria	RR 1.78 (1.47, 2.14)	219	[178]
Risk factors for composite poor renal outcome			
Older age at onset	WMD 1.77 (0.35, 3.18)	969	[108][a]
Low eGFR as initial symptom	WMD -23.93 (−33.78, −14.09)	969	[108][a]
Higher degree of proteinuria	HR 1.20 (1.06, 1.35)	275	[29][b]
Nephrotic syndrome at onset	OR 1.74 (1.12, 2.70)	969	[108][a]
Nephritic-nephrotic syndrome at onset	OR 4.55 (2.89, 7.15) RR 11.9 (4.1, 41.5)	969 1133	[108][a] [179][c]
Crescents	OR 3.85 (2.37, 6.28)	969	[108][a]
S1 Glomerulosclerosis	HR 4.09 (1.11, 15.03)	275	[29][b]
M1 Mesangial hypercellularity	HR 4.02 (1.22, 13.30)	113	[105][d]
T1/2 Significant tubulointerstitial fibrosis	HR 12.28 (2.72, 55.27)	113	[105][d]

[a] Poor renal outcome defined as hypertension, proteinuria >40 mg/m^2/h, increased serum creatinine, uremia, ESKD, dialysis, or transplantation
[b] Poor renal outcome defined as 30% decrease in baseline eGFR, doubling of serum creatinine, or ESKD
[c] Poor renal outcome defined as nephrotic syndrome, nephritis, renal failure (serum creatinine above upper limit of normal), or hypertension
[d] Poor renal outcome defined as doubling of serum creatinine or ESRD
CKD chronic kidney disease; ESKD end kidney renal disease; IF/TA interstitial fibrosis/tubular atrophy; S1 any segmental glomerulosclerosis; M1 mesangial hypercellularity $\geq$50% of glomeruli; T1/2 significant tubule-interstitial fibrosis $>$25%, $>$50%, respectively; RR relative risk; WMD weighted mean difference; OR odds ratio; HR hazards ratio

kidney disease, dialysis, or transplantation [108]. A study of 219 biopsy-proven pediatric and adult cases of IgA vasculitis with nephritis identified persistent proteinuria at 1 year as a risk factor for doubling of serum creatinine (RR 1.44; 95% CI 1.27, 1.64) and end stage kidney disease (RR 1.78; 95% CI 1.47, 2.14). This study also identified that moderately impaired eGFR (eGFR 60–90 mL/min/1.73 m^2 in children or serum creatinine 1.5–3 mg/dL in adults) at presentation was associated with increased risk of doubling of serum creatinine at follow-up (RR 1.97; 95% CI 1.02, 3.78) [178]. Biopsy findings provide limited information regarding renal prognosis, as discussed above.

End Stage Kidney Disease

End stage kidney disease is an important long term outcome, but is infrequent. A retrospective study from Japan on 1120 children with IgA vasculitis found that 1.2% progressed to end stage kidney disease [35]. This finding was similar to a systematic review on 1133 children with IgA vasculitis that showed that 1.8% (95% CI 1.1, 2.8) of patients with IgA vasculitis are likely to progress to ESKD [179]. Kidney histology including crescents in over 50% glomeruli, interstitial fibrosis over 25%, mesangial hypercellularity and segmental glomerulosclerosis, nephrotic range proteinuria at presentation, and persistent proteinuria 1 year from onset of illness is associated with ESKD (Table 3).

Recurrence in Renal Allograft

In IgA vasculitis with nephritis, clinical recurrence in renal allografts is not uncommon; recurrence of mesangial IgA deposits is frequent [179–184]. Histologic recurrence is not necessarily associated with clinical recurrence. Some reports suggest that transplantation from living-related donors was associated with an increased risk of recurrence of the disease [184–186]. However, the association of living donation with recurrence of the disease and its effect on graft survival is not clear [186]. Data from the US national registry on organ transplantation showed histologically proven recurrence of IgA vasculitis in 13.6% of 339 transplant recipients with the disease. However, the 5- and 10-year allograft survival was not different between transplant recipients with IgA vasculitis compared with other conditions [185].

Although IgA vasculitis with nephritis in children is considered a self-limiting disease, a proportion of patients may show hypertension, chronic kidney disease, and ESKD, emphasizing the need for long-term follow-up [96, 178, 185].

Conclusions

See Table 3

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Renal Vasculitis

19

Seza Ozen, Diclehan Orhan, and Stephen D. Marks

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Abstract

The nomenclature of vasculitides has been revised in light of recent understanding of the pathogenesis of these diseases. The Ankara 2008 childhood classification criteria aid in the management of pediatric patients with vasculitis. The most common childhood vasculitis, IgA vasculitis (Henoch-Schönlein purpura) is reviewed elsewhere. The other common vasculitis of childhood, Kawasaki disease affects medium-sized arteries but rarely the kidney.

ANCA-associated vasculitis (AAV) often affects the kidney, mainly in form of pauci-immune necrotizing glomerulonephritis. The most common AAV is granulomatosis with polyangiitis (Wegener's granulomatosis). Polyarteritis nodosa (PAN) is a medium-sized arteritis that may involve the arteries of the kidneys as well. Deficiency of adenosine deaminase (ADA2) might mimic PAN and should be considered in patients with features of PAN with family history, or those with stroke or with hematological and/or immunological abnormalities. Takayasu arteritis and rarely Behçet disease can also affect the vessels of the kidney. Recent management and treatment strategies guide us in the care of these children.

Keywords

ANCA-associated vasculitis · child · IgA Vasculitis · Kawasaki disease · Polyarteritis nodosa · Deficiency of adenosine deaminase · Takayasu arteritis

Synonyms

GPA: Wegener Granulomatosis; EGPA: Churg Strauss Syndrome

Introduction

The vasculitides are a group of rare diseases that involve inflammation in walls of blood vessels. This inflammation leads to narrowing or aneurysm of the vessel resulting in protean systemic manifestations. The kidney is a frequently affected target organ.

Vasculitides have predilections for the size of the vessel and the organ they affect. A number of attempts have been made to classify vasculitis syndromes based on these features. Recently the nomenclature criteria suggested by the Chapel Hill Consensus Conference (CHCC) has been revised [1]. The small vessel involvement is now divided into two main groups based on the pathogenesis of these diseases (Table 1). Specific vasculitis eponyms have been replaced by suitable descriptive terms with pathophysiologic specificity where feasible [1]. Some eponyms are retained; another novelty is the addition of new categories:

- Variable vessel vasculitis includes vasculitides that may affect any vessel size, such as Behçet disease
- Single vessel vasculitis includes isolated vasculitides, such as central nervous system vasculitis
- Vasculitis associated with systemic disease such as systemic lupus erythematosus
- Vasculitis with probable etiology, for example, hepatitis associated, monogenic diseases with vasculitis.

The aforementioned CHCC are mainly definition criteria. Almost three decades earlier, the American College of Rheumatology had suggested classification criteria for adults for each vasculitis [2–4]. Therefore, in 2006 a group of pediatric rheumatologists and nephrologists published the classification criteria for the common childhood vasculitides with names at that time of Henoch-Schönlein purpura, Kawasaki disease, polyarteritis nodosa, Wegener granulomatosis, and Takayasu arteritis [5]. These criteria have been revised and validated and published as the Ankara 2008 criteria, endorsed by EULAR (European League Against Rheumatism), PRINTO (Pediatric Rheumatology International Trials Organization), and PRES (Pediatric Rheumatology European Society) [6]. One should remember that all the mentioned criteria are classification criteria and the

Table 1 New classification of childhood vasculitides. (Modified from Ref. [1])

Large-vessel vasculitis
Takayasu arteritis (TAK)
Giant cell arteritis (GCA)
Medium-vessel vasculitis
Polyarteritis nodosa (PAN)
Kawasaki disease (KD)
Small-vessel vasculitis
Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV): Microscopic polyangiitis (MPA); granulomatosis with polyangiitis (Wegener, GPA); Eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome, EGPA)
Immune complex vasculitis: Anti-glomerular basement membrane (anti-GBM) disease; cryoglobulinemic vasculitis; IgA vasculitis (Henoch-Schönlein); hypocomplementemic urticarial vasculitis (anti-C1q vasculitis)
Variable vessel vasculitis
Behçet disease
Cogan syndrome
Single-organ vasculitis
Cutaneous leukocytoclastic angiitis
Cutaneous arteritis
Primary central nervous system vasculitis
Isolated aortitis
Vasculitis associated with systemic disease
Lupus vasculitis
Rheumatoid vasculitis
Sarcoid vasculitis
Vasculitis with probable etiology
Hepatitis C associated cryoglobulinemic vasculitis
Hepatitis B associated vasculitis
Syphilis-associated aortitis
Drug-associated immune complex vasculitis
Drug-associated ANCA-associated vasculitis
Cancer-associated vasculitis
Others

diagnosis of these diseases depend on the judicious assessment of the patient.

The most common vasculitides of children are IgA vasculitis (Henoch-Schönlein purpura) and Kawasaki disease (KD). The former is reviewed in a separate chapter. The remaining primary vasculitides that affect the kidney will be reviewed.

ANCA Associated Vasculitides

Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitides (AAV) are a group of disorders that can cause a rapidly progressive glomerulonephritis with chronic, often relapsing disease, which can be organ or life threatening with high morbidity and mortality despite immunosuppression. There are three main conditions characterized by necrotizing inflammation of small to medium vessels in association with autoantibodies against the cytoplasmic region of the neutrophil (ANCA), including the commonest condition, granulomatosis with polyangiitis (GPA; formerly Wegener granulomatosis), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA; formerly Churg-Strauss syndrome). Children with all three conditions present with rapidly progressive glomerulonephritis associated with necrotizing glomerulonephritis (capillaritis) and is characteristically pauci-immune (few or no immune deposits, affecting small vessels, i.e., capillaries, venules, or arterioles) [1]. Renal limited vasculitis is a term used for renal histopathology (previously classified as pauci-immune, necrotizing crescentic, or ANCA-associated glomerulonephritis) in the absence of vasculitis in other organs.

Approximately 80% patients with renal limited vasculitis are positive for ANCA, predominantly myeloperoxidase (MPO-ANCA), and thus may be cohorted with patients diagnosed as MPA. These disorders are associated with either proteinase-3 (PR3) or MPO-ANCA. However, MPO is more often associated with microscopic polyangiitis, whereas PR3-ANCA is more common in granulomatosis with polyangiitis.

Microscopic Polyangiitis

Necrotizing glomerulonephritis is frequently seen with necrotizing arteritis involving small and medium-sized arteries and pulmonary capillaritis [1]. The presence of ANCA directed often against the MPO antigen, shown by ELISA and/or strong perinuclear staining pattern by indirect immunofluorescence, has been added to the definition in children [5, 6].

Pathogenesis: Both in vitro and in vivo experimental data support the pathogenic role of ANCA in MPA [7]. In fact, a mice model has introduced direct role of the injury by anti-MPO: Anti-MPO IgG led to focal necrotizing and crescentic GN with paucity of glomerular immune deposition, which mimics the human disease [8]. However, we still need further data on the factors that are effective in production of ANCA in the first place, and the genetic factors involved.

A recent genome wide association study has provided important information [9]. This study showed that the pathogenesis of AAV has a genetic component and shows genetic distinctions between GPA and MPA that are associated with ANCA specificity. Anti-MPO-ANCA, which is more common in MPA, was associated with certain SNPs in HLA-DQ [10].

Clinical and laboratory features: The presence of histopathologically proven necrotizing glomerulonephritis along with a positive ANCA (often MPO-ANCA) titer in the absence of granulomatous lesions and upper respiratory tract involvement leads to a diagnosis of MPA [6]. Childhood cases have been reported as small series [11–15]. Hattori et al. [13] have reviewed 34 ANCA-seropositive Japanese pediatric patients with biopsy-proven pauci-immune necrotizing crescentic glomerulonephritis and 21 were classified as MPA with disease similar to that of adults except for female predominance. Patients who subsequently developed end-stage kidney disease (ESKD; $n = 9$) had significantly worse renal function and more chronic pathological lesions at diagnosis compared with patients with favorable renal outcome [13]. Forty-eight pediatric MPA patients were also subsequently described with similar features [11].

In a series reported by Peco-Antic et al., 57% patients had pulmonary-renal syndrome, one developing chronic kidney disease and two with acute kidney injury progressed to ESKD [15]. In another series of six pediatric patients from India, three had the pulmonary renal syndrome [12]. We reported a series of 25 patients with PAN, 10 of whom were classified as small vessel necrotizing arteritis (MPA) [16]. Six developed renal dysfunction, three of them had pulmonary-renal syndrome and all had high MPO-ANCA levels. Six had percutaneous renal biopsies showing pauci-immune, necrotizing crescentic glomerulonephritis, and four (40%) progressed to chronic kidney disease [16].

Laboratory investigations: An immunofluorescence testing for ANCA often reveals a p-ANCA pattern. Further ELISA testing should also be ordered which typically will show a high titer of MPO-ANCA [6, 17]. Urinalysis will reflect the glomerular disease with proteinuria, hematuria, and an active urine sediment with casts. Renal function tests were impaired in 30–60% of patients reported in a pediatric series [18, 19]. A chest x-ray is required in all patients and in patients with abnormalities, then chest CT is warranted to define the extent of lung involvement.

Pathology: Percutaneous renal biopsy is indicated in children with renal involvement. MPA is characterized by necrotizing vasculitis with few or no immune deposits, affecting capillaries, venules, or arterioles [7]. All arterial lesions are usually at the same stage. Kidneys show necrotizing glomerulonephritis, which is focal and segmental to severe diffuse and crescentic (Fig. 1). No immune deposits are detected on immunofluorescence or electron microscopy. Sausage

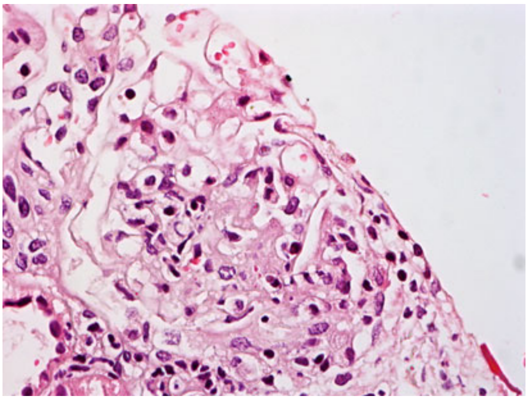

Fig. 1 Microscopic polyangiitis with segmental necrosis and proliferation of parietal cells of the Bowman's capsule in the periphery of the glomerulus

shaped microaneurysms of interlobular arteries are also reported [15, 20–22].

Differential diagnosis includes pauci-immune glomerulonephritis including EGPA, severe cases of IgA vasculitis, and coagulation disorders. Etiologies of necrotizing and crescentic glomerulonephritis, pauci-immune glomerulonephritis, are generally best differentiated clinically, and not histologically [20, 23].

Treatment and Prognosis: The prognosis of AAV in children with MPA, GPA, and EGPA has substantially improved with results of controlled studies in adults and new treatment modalities. EUVAS (European Vasculitis Study group) has carried out multicenter, controlled studies that have provided us evidence for treatment of these patients. Treatment for AAV can be divided into two parts, induction and maintenance.

For induction treatment, patients should receive corticosteroids and cyclophosphamide. In patients with severe disease, three daily pulse of intravenous methylprednisolone is suggested to be continued with oral corticosteroids. Daily oral prednisolone (1–2 mg/kg/d) and intravenous cyclophosphamide pulses (500–750 mg/m^2, reduced to 250 mg/m^2 if renal dysfunction; every 2–4 weeks) is initiated as induction therapy. Whether cyclophosphamide is to be given via an oral or intravenous route is a matter of debate. The CYCLOPS trial in adults showed that pulse cyclophosphamide may be associated with a higher relapse risk than oral therapy [24]. However, there was no difference in renal function at a median follow-up of 4.3 years ($P = 0.82$). Other agents employed in remission induction include anti-TNF therapy and MMF. Increasing consideration is being given to the use of rituximab for severe or relapsing disease, particularly for those with concerns regarding glucocorticoid or cyclophosphamide toxicity.

For maintenance treatment, the prednisolone dose is tapered following clinical and laboratory response, but not before 4 weeks. A suggested wean is to reduce prednisolone dose by 10 mg every 2 weeks, to reach a minimum of 5–10 mg/d and subsequently on alternate days, which may be continued for a year after clinical remission. For maintenance regimen, the total cyclophosphamide dose has been a concern for pediatricians. The CYCAZAREM trial showed that the replacement of cyclophosphamide with azathioprine at 3 months is effective for disease control [25]. Methotrexate has been shown to be an alternative for maintenance treatment especially for non-renal manifestations [26]. Another randomized trial that compared mycophenolate mofetil (MMF) to azathioprine for remission maintenance in AAV showed that the latter was less effective than the former in maintaining remission [27].

Plasmapheresis, in addition to immunosuppressive treatment, may be used for life-threatening disease. The previous MEPEX study has shown that plasmapheresis is advantageous over pulse corticosteroids in patients with renal dysfunction [28]. However, the new PEXIVAS study has not confirmed these results.

Two controlled studies in 2010, one from Europe and the other from North America, confirmed the efficacy of anti-B cell therapy in patients with AAV [29, 30]. These two studies compared rituximab and cyclophosphamide for the induction of remission with different protocols. There were no significant differences in remission rates nor adverse events. Intravenous rituximab is now a safe alternative for adult patients who are resistant or intolerant to cyclophosphamide [29, 30]. A pediatric study is underway. For the time being rituximab should be considered especially in children with relapsing disease who have already received cyclophosphamide.

Granulomatous Polyangiitis (GPA) (Wegener Granulomatosis)

GPA is defined as a necrotizing granulomatous inflammation of the upper and lower respiratory tract, necrotizing vasculitis affecting small to medium-sized vessels and glomerulonephritis [1]. GPA is a vasculitides with major renal involvement like MPA. Children frequently have constitutional symptoms, such as fever, anorexia, and weight loss with multiorgan or generalized disease. According to the Ankara 2008 criteria revised for childhood, three of the following six should be present to classify as GPA [6]:

- Renal involvement
- Upper airway involvement (70 to 100%) with recurrent epistaxis or sinusitis
- Laryngo-tracheo-bronchial stenosis
- Pulmonary involvement (chest x-ray or CT showing diffuse pulmonary infiltrates, pulmonary nodules, cavitating lesions, and granulomata without cavitation)
- Histopathology (granulomatous inflammation)
- Positive ANCA

Pathogenesis: The etiology of GPA is not known; the endothelium itself is the target for initial injury. Studies suggest a direct involvement of ANCA in the pathogenesis of the disease; inflammatory cells primed with ANCA are cytotoxic to endothelial cells [31, 32]. It is known that in vitro ANCA induces endothelial injury through activation of neutrophils and the reactive oxygen molecules, proteases, and cytokines that they release. ANCA is “active” against the proteinase 3 (PR3) on neutrophils in GPA. In vitro ANCA can further activate neutrophils to release reactive oxygen species, which in conjunction with neutrophils may damage and result in inflammation of endothelial cells [7]. There is a convincing mouse model defined with MPO-ANCA, mimicking the human disease; however, the same results were not obtained with PR3-ANCA [7].

There have been a number of genetic associations with the disease including HLA antigens, a region on chromosome 6p21.3 [9]. Recently a genome wide association study (GWAS) in PR3 and MPO-ANCA associated AAV patients has provided us further important information: In GPA, anti-proteinase 3 ANCA was associated with HLA-DPB1 and the genes encoding α(1)-antitrypsin (SERPINA1) and proteinase 3. Polymorphisms of the natural inhibitor of PR3, alpha-1-antitrypsin, had been previously implicated for increased risk and morbidity of GPA. This study suggested that the response against PR3 is a pathogenic feature of GPA [9].

Clinical and laboratory features: Data from small pediatric series reflect that 90% of children with GPA present with symptoms related to the upper and lower respiratory tract vasculitis, sinusitis, epistaxis, and nasal inflammation [33–38]. Lower-airway inflammation manifests as cough, dyspnea and hemoptysis; 30% have chest x-ray abnormalities [33–35, 38]. Among 25 patients reported by Akikusa et al., renal involvement was present in 22, and upper airway involvement in 21 patients at presentation and 24 during follow-up [33]. Twenty patients had initial pulmonary involvement, most commonly nodules (44%) and pulmonary hemorrhage (44%), and four (16%) had venous thrombotic events [33].

Renal involvement occurs in 10 to 100% of affected children and contributes to significant morbidity [33–35, 38]. Patients present with urinary sediment abnormalities or mild renal dysfunction and glomerulonephritis diagnosed by biopsy is common. In the aforementioned series of 25 children, glomerulonephritis was present in 22 patients at presentation of disease [33]; only one of 11 patients who presented with or developed renal dysfunction had normalization of serum creatinine [33]. Necrotizing glomerulonephritis is one of the most serious manifestations in GPA associated with renal dysfunction [33].

Clinical findings include blurred vision, eye pain, conjunctivitis, episcleritis, and otitis media [33–35, 38]. Neurological involvement presents as cranial nerve palsies, seizures, or neuropathies [32]. Skin lesions include palpable purpura, nodules, ulceration, and gangrene. Musculoskeletal symptoms may be present but cardiac involvement is rare.

A cytoplasmic pattern on immunofluorescent staining of ANCA (c-ANCA) is present in 70–90% of patients with active GPA [1, 33, 35]. ELISA tests detecting PR3-ANCA confirm this specificity in a high proportion of patients. Investigations show elevated white blood count and acute phase reactants. Urinalysis shows hematuria, proteinuria, and casts when there is renal involvement. Chest x-ray and pulmonary function tests are indicated for assessment of lung disease [6, 32]. The chest x-ray may show nodular infiltrates and nodules that might require assessment with chest CT.

Pathology: The typical histologic lesion of granulomatous polyangiitis is necrotizing vasculitis of small arteries and veins, usually with granuloma formation (Fig. 2). Granulomas may be intravascular or extravascular. Multiple organ systems can be involved. Biopsy of paranasal sinus, nasopharyngeal, or tracheal lesions reveal acute or chronic inflammation or frank vasculitis with or without granulomas. Pulmonary lesions microscopically show granulomas and vasculitis. Renal involvement is observed as necrotizing glomerulonephritis, often with necrosis, cellular crescents, and usually with glomerular thrombosis. Interstitial inflammation is common, and renal papillary necrosis is seen in ~20%. Unlike the biopsy of lung or sinuses, the renal biopsy rarely shows a granuloma [1, 7, 23, 31, 32].

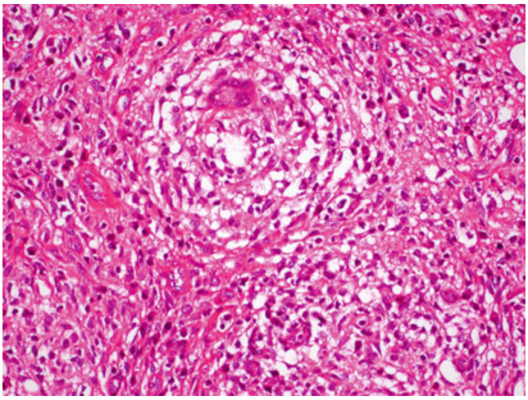

Fig. 2 GPA/WG. Necrotizing granulomatous inflammation of the vessel with multinucleate giant cells

Differential diagnosis is similar to MPA, but in addition, other granulomatous diseases such as sarcoidosis, lymphomatoid granulomatosis, and tuberculosis are also included.

Treatment and Outcome: Treatment of GPA and MPA is similar. In fact, most of the adult studies published by EUVAS include MPA and GPA patients together [26–28, 39, 40]. Rituximab has been shown to be effective and is an increasingly used form of induction and maintenance therapy [30, 41]. Relapses are frequent as in the adults. Treatment with cotrimoxazole may help reduce the frequency of relapses. The overall data support the prognostic impact of renal function at diagnosis and of renal relapses during follow-up.

Eosinophilic Granulomatosis with Polyangiitis (EGPA) (Churg-Strauss Syndrome)

EGPA is very rare in childhood and accounts for ~2% children with primary systemic vasculitis. EGPA is a necrotizing vasculitis of small and medium-sized vessels that predominantly affects the upper airway and lung. EGPA is defined as a systemic necrotizing vasculitis with hypereosinophilia and extravascular granulomas along with asthma [1, 42]. Patients present with history of asthma and allergy, involvement of paranasal sinuses and peripheral nervous system, and eosinophilia. Skin involvement and vasculitis is similar to PAN. However, this pauci-immune small vessel vasculitis is distinguished from GPA and MPA by the presence of granulomatous inflammation and asthma with hypereosinophilia. Among adult patients, ~20% show glomerulonephritis and renal dysfunction [43].

The laboratory work-up is similar to MPA or GPA but the presence of ANCA is less common in children (0 to 25%). Typically, p-ANCA pattern is expected but specific laboratory tests are required for the diagnosis and differential diagnosis.

Pathology: The classic findings of EGPA are eosinophil-rich, granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small to medium-sized vessels

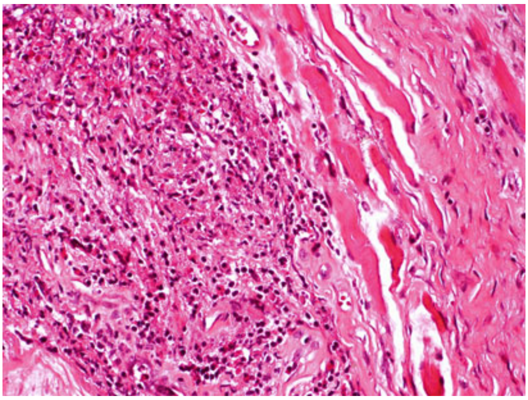

Fig. 3 EGA/CSS. Granulomatous vasculitis with multinucleate giant cell and eosinophils

(Fig. 3). Renal manifestations include pauci-immune crescentic glomerulonephritis, renal arteritis, arteriolitis, and medullary angiitis. Microscopic features on renal biopsy include necrotizing and crescentic glomerulonephritis without immune complex deposition, fibrinoid necrosis of arteries, and interstitial nephritis with eosinophilia [7, 44].

Treatment of EGPA is similar to that of other AAV.

Kawasaki Disease

Definition and classification: Kawasaki disease is a vasculitis affecting the skin and mucosa, characterized by preference for the coronary vessels. According to the Chapel Hill criteria, Kawasaki disease is defined as arteritis involving large, medium-sized, and small arteries and associated with mucocutaneous lymph node syndrome [1]. The disease is classified based on presence of fever of at least 5 days duration, plus four of the following five features [5, 45, 46].

- Changes in peripheral extremities (edema and/or erythema of the hands and feet and subsequent periungual desquamation), or desquamation of the perineal area (Fig. 4)
- Polymorphous exanthema mainly truncal
- Bilateral non-purulent conjunctival injection
- Changes of lips and oral cavity: injection of oral and pharyngeal mucosa
- Cervical lymphadenopathy, usually unilateral

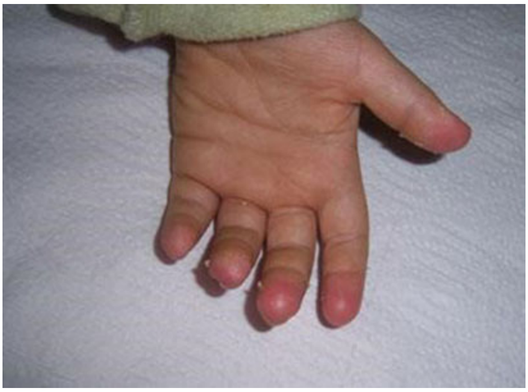

Fig. 4 Peeling in the hands of a patient with Kawasaki disease

Pathogenesis: As is the case of many vasculitides, Kawasaki disease is an abnormal immune response to environmental or infectious trigger(s), in genetically predisposed individuals. Superantigens have been frequently implicated as the causative agents. This is supported by the skewing of the T-cell receptor Vβ repertoire [46]. The fact that Kawasaki disease mainly affects young children may be due to the timing of the exposure of the environmental agent in a naïve immune pathway.

Clinical features: The clinical features of the disease are as above [45, 46]. Although renal involvement is rare, nephrologists should be aware of the disease spectrum and rare involvement of renal vessels. Pyuria and urethritis are frequent [46, 47]. Vasculitis might rarely affect the renal arteries, manifested as renal artery aneurysms [48]. Angiographic evaluation of other mid-size arteries should be done if there are suggestive features, such as hypertension. Renal parenchymal involvement and acute kidney injury have been reported only in rare case reports, including acute interstitial nephritis [49].

As for laboratory investigations, a full blood count is mandatory. Thrombocytosis is common at the second week of the disease; however, platelet counts may be low in the early phase of the disease [46]. Acute phase reactants are high. Every child suspected of having KD should have an echocardiography to investigate for coronary artery involvement.

Pathology: Systemic vasculitis is the characteristic pathological finding in Kawasaki disease [50, 51]. In addition to vasculitis and inflammatory lesions in various organs and the heart, interstitial nephritis and cystitis have been reported [50–52]. Histologically, coronary arteritis begins 6–8 days after onset of the disease. The inflammation is composed of neutrophils, monocytes, and macrophages in all layers of the artery [50–53]. Arterial dilatation begins, and progressive destruction of the adventitia causes saccular aneurysms. These aneurysms can thrombose or rupture [50–53]. Varying incidence of panarteritis in kidneys of Kawasaki disease is reported [52, 53]. Asaji et al. reported that 73% patients had features of angiitis or scars in their kidney. Arteritis and granulomatous inflammation were noted in patients who died within 1 month after the onset of KD. Arteritis in the kidney was milder than coronary arteritis, and developed several days later than the coronary arteritis. Panangiitis was localized to the interlobar arteries and rarely seen in arcuate and intralobular arteries [52]. Glomerular changes, such as segmental or global glomerulosclerosis and focal segmental proliferation, and intracapillary proliferative glomerulonephritis are reported [52, 53].

Treatment and Prognosis: Recently experts have participated in the European Single Hub and Access point for pediatric rheumatology in Europe initiative (SHARE) for recommendations for therapy for Kawasaki disease [54]. Treatment includes intravenous immunoglobulin (IVIG) administered at 2 g/kg along with salicylates at a dose of 30–80 mg/kg/day [46, 48, 54]. Aspirin should be given for 6 weeks or until the ESR and platelet counts normalize and lifelong for those who have any cardiac involvement [46, 48, 54]. SHARE recommendations also suggest to give corticosteroids to patients with severe illness, including those who are IVIG resistant, those with features of shock and those with coronary aneurysms [54]. Biologics should be considered in severe and steroid resistant cases as well.

Kawasaki disease is the top cause of acquired heart disease in the western world [46]. Therefore, early recognition of the disease and administration of IVIG in the first 10 days of initiation is crucial. The follow-up of patients with coronary involvement should always include a pediatric cardiologist. The prognosis is excellent in patients treated within the first days of their disease, since Kawasaki disease is self-limiting if the coronaries are spared.

Childhood Polyarteritis Nodosa (PAN)

Definition and Classification: The Chapel Hill Nomenclature criteria 2012 defines polyarteritis as necrotizing arteritis of medium or small arteries *without* glomerulonephritis or vasculitis in arterioles, capillaries, or venules, and not associated with circulating ANCA [1].

Childhood PAN differs from microscopic polyangiitis (MPA) through the absence of necrotizing glomerulonephritis and absence of ANCA. Renal involvement in childhood PAN occurs through necrotizing arteritis of the renal vascular bed, whereas the presence of necrotizing glomerulonephritis classifies the child as MPA [1]. The renal features are related to the involvement of the medium sized vessels in the kidney. In a large adult series, Agard et al. reported that the initial manifestations of both classic PAN and MPA were similar, except for gastrointestinal involvement and peripheral neuropathy being more frequent in PAN [18].

The Ankara 2008 pediatric criteria for the classification of polyarteritis nodosa (PAN) have been validated (Table 2) [6]. The new criteria of childhood PAN include the demonstration of necrotizing arteritis in small and medium size arteries or aneurysms in medium-sized arteries by imaging techniques, which reflects the necrotizing inflammation in these vessels [6].

Pathogenesis: As in Kawasaki disease, infections are implicated as a triggering agent in a predisposed host. In fact, a number of reports point to the role of streptococci in childhood PAN, especially in cutaneous PAN [55]. On the other hand, other forms of PAN have more specific pathogenesis: hepatitis B related PAN is an immune complex disease and is now classified as "vasculitis associated with probable etiology" [1]. This association has declined with recent

Table 2 EULAR/PRINTO/PRES (Ankara 2008) classification criteria for childhood PAN [6]

Evidence of necrotizing vasculitis in medium or small arteries or an angiographic abnormality showing aneurysm, stenosis, or occlusion of a medium- or small-sized artery (histopathology or angiography mandatory), plus one out of five of the following criteria:
1. Skin involvement (livedo reticularis, tender subcutaneous nodules, other vasculitic lesions)
2. Myalgia or muscle tenderness
3. Systemic hypertension (systolic or diastolic blood pressure greater than 95th percentile for age, sex, and height centile)
4. Peripheral neuropathy (sensory peripheral neuropathy, or motor mononeuritis multiplex)
5. Renal involvement: Proteinuria (>0.3 g/24-h proteinuria or urine albumin: creatinine ratio >30 mg/mmol on spot morning sample; ≥2+ protein on dipstick); hematuria or red cell casts (>5 red cells/high power field; red blood cells casts in urinary sediment); or impaired renal function (measured or calculated glomerular filtration rate <50 ml/min/1.73 m^2)

vaccination protocols. Recently, an association of childhood PAN with pathogenic variants in *MEFV* (familial Mediterranean fever) has been shown in Turkish children [56, 57].

Clinical features: PAN presents with general findings and wide range of symptoms. The disease often has an insidious onset with constitutional symptoms [6, 58]. Children with PAN present with constitutional symptoms, skin manifestations, myalgia, and organ involvement. In an effort to define the characteristics of childhood PAN throughout the world, we recruited 110 patients from 21 pediatric centers from over the world [58]. The mean patient age was 9.1 ± 3.6 years (range 1–16 years), with equal number of girls and boys. Features were similar to those reported in small series and were different from adult data. The mean patient age was 9.3 and 7.5 years in two other series, both including infants [59, 60]. In the aforementioned multinational study, fever (87%), myalgia (83%), and skin (88%) manifestations were the leading features, whereas renal involvement was reported in 19% [58]. Pulmonary and cardiac disease was reported in 11% and 14%, respectively, with peripheral neuropathy and CNS involvement seen in few patients. Similar frequencies of clinical features were reported in smaller studies from India and Croatia [59, 60]. A number of patients do not have typical presentations of classic PAN, but have inflammation in arteries of varying size in organ systems. Erden et al. have compared the features of PAN in children and adults from a tertiary center [61]. Joint and skin involvement were more common in children (86.7% vs. 59.1%; 93.3% vs. 72.7%, respectively), while renal and neurologic involvement were more frequent in adult patients (50% vs. 20%; 59.1% vs. 40%, respectively; all $P > 0.05$) [61].

Renal involvement presents with systemic vasculitis along with an ischemic kidney due to the inflammation of the medium-sized arteries [6, 62]. The glomerular capillaries are not affected. Renal involvement in childhood PAN may present as hypertension reflecting the necrotizing vasculitis of the renal arteries. In fact, in the series of 110 children with PAN, 43% of patients with the systemic disease had hypertension. The diagnosis was confirmed by angiography in 52.5% patients [58].

Full blood count may reveal anemia, leukocytosis, and thrombocytosis. The work-up should include urinalysis, monitoring renal function, acute phase reactants for disease activity, and necessary imaging [63]. Imaging of mid-sized arteries is required to search for aneurysm in a patient who presents with hypertension or ischemic abdominal pain. In a review of the angiograms in PAN patients, Brogan et al. concluded that the most reliable non-aneurysmal findings were perfusion defects, presence of collateral arteries, lack of crossing of peripheral renal arteries, and delayed emptying of small arteries [62]. Children with medium or large vessel aneurysms or those with renal perfusion defects may have impaired kidney function. In patients without hypertension, renal involvement is unlikely and mesenteric or hepatic arteritis may be the cause of abdominal pain and ischemia. CT angiography may be sufficient for the diagnosis of PAN, although not as sensitive as a classic angiogram.

Targeted tissue biopsy is indicated, although percutaneous renal biopsy is usually not indicated since the glomerular capillaries are spared. The diagnosis of PAN can be confirmed by histopathological demonstration of necrotizing vasculitis and/or radiologic demonstration of aneurysms and other angiographic typical findings.

The differential diagnosis depends on the spectrum of organ involvement. Other forms of vasculitis and secondary vasculitides need to be considered. Infections such as bacterial endocarditis with microembolism as well as autoinflammatory syndromes may mimic features of PAN. Pathogenic variants in *ADA2,* encoding adenosine deaminase 2 results in a syndrome of intermittent fevers, early-onset lacunar strokes and neurovascular manifestations, livedoid rash, hepatosplenomegaly, and systemic polyarteritis nodosa like vasculopathy, have been described [64].

Pathology: PAN is characterized by necrotizing inflammation throughout the wall of small to medium-sized arteries [1, 12, 17, 19, 65]. The kidney, gastrointestinal tract, liver, skin, pancreas, muscles, and other sites can be involved. Acute lesions are characterized by fibrinoid necrosis of the arterial wall with inflammation (Fig. 5). Fibrinoid necrosis may be circumferential, but is often localized to a single artery segment. Inflammation is composed of neutrophils early in the disease, and later, lymphocytes, plasma cells, and macrophages (Fig. 6). Healing lesions show marked fibrous thickening of the arterial wall with fragmentation of the elastic lamina. Individual vessels reveal different stages of the inflammatory process [17, 19, 65].

Intravascular thrombosis is a frequent sequel to the vasculitis leading to ischemia and infarction in the areas supplied by these vessels. Necrosis of muscle in vessel wall may lead to focal aneurysm as a late complication. Necrotizing vasculitis of renal, interlobar, and arcuate arteries at branch points with aneurysmal dilatation, fibrinoid necrosis, and neutrophilic infiltration of vessel wall are observed in the kidney. Later, perivascular tissue and media shows mononuclear infiltration and fibrosis, and recanalization of the thrombosed vessel is seen. Glomerular lesions (ischemic collapse and sclerosis) are not specific to PAN [1, 12, 65].

Treatment and Prognosis: Treatment of systemic PAN is based on uncontrolled pediatric data and recommendations on patients with adult-onset disease. There is need for prospective multicenter studies to define the efficacy and duration of immunosuppressive treatment. A group of experts recently proposed recommendations for treatment of systemic vasculitis, including PAN [63]. According to these recommendations, management of systemic vasculitis requires induction treatment, followed by less intense maintenance therapy. The former may include corticosteroids and intravenous cyclophosphamide [63]. For maintenance treatment, azathioprine, mycophenolate mofetil, and methotrexate are first-line agents;

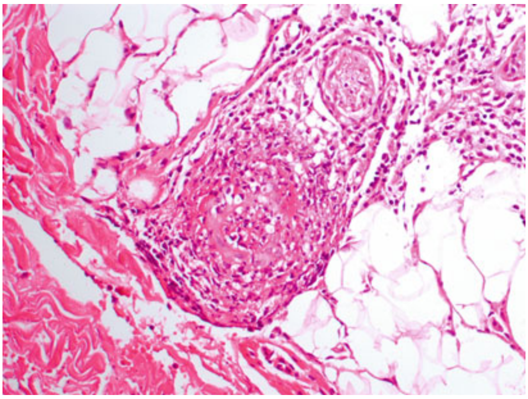

Fig. 5 PAN. Fibrinoid necrosis and inflammation in a medium-sized artery wall

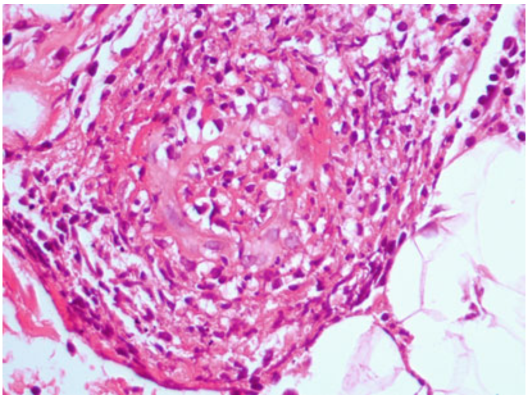

Fig. 6 PAN. Fibrinoid necrosis with neutrophilic infiltration in vessel wall

biologics may be considered for recalcitrant disease [63]. Maintenance treatment is advised for 12–36 months, depending on course of the disease. The prognosis of PAN has improved in recent years due to timely diagnosis and effective therapy.

Deficiency of Adenosine Deaminase 2 (ADA2)

Definition: Deficiency of ADA 2 is a monogenic form of vasculitis [64, 66]. The disease is due to mutations in the gene *ADA2* or *CECR1* encoding ADA2 [64–67], leading to disease mimicking PAN, with inflammation of medium-sized arteries in the kidney and elsewhere.

Pathogenesis: ADA2 is highly expressed in myeloid cells and produced by activated monocytes, macrophages, and dendritic cells [65–67]. The inflammatory component of the disease is thought to be due to monocyte-macrophage polarization toward the M1 (inflammatory) subset [65, 67]. M1 macrophages are known to promote inflammation and tissue damage. ADA2 appears to be critical for the maintenance of vascular integrity and ADA2 deficiency is thought to disrupt endothelial cells, which may explain the vasculitic and neurologic component of the disease. Livedoid rash and other types of nonspecific skin rash such as nodular rash and macular erythema are also described [65–67].

Clinical features: Clinical presentation and age of onset vary widely. Medium-sized arteries of any organ system can be affected [64, 65]. Necrotizing inflammation of renal arteries results in microaneurysms or stenosis on CT angiography. Other features include livedoid rash and early cerebrovascular accidents [64–67]. DADA2 patients also display features of clinical inflammation such as low-grade fever or high CRP. Cutaneous manifestations are the most common feature of deficiency of ADA2, present in three-fourths of patients [64–67].

Of all patients, 50% have one or more neurological events. MRI shows acute or chronic lacunar ischemic infarcts located in the deep-brain nuclei and/or the brain stem, with sparing of subcortical white matter. Onset of ischemic strokes can be in infancy; hemorrhagic stroke and intracranial bleeding may also occur [67]. Hematological involvement may be present in the form of varying degrees of cytopenias [68]. We have previously reported that patients with deficiency of ADA2 may not have the thrombocytosis of classic PAN, and instead show thrombocytopenia [65]. Hypogammaglobulinemia is not rare, and other mild immunological abnormalities and fluctuating titers of autoantibodies may be detected [64, 67].

Red flags for the diagnosis of ADA2 deficiency include a family history of similar disease, and/or early cerebrovascular accidents, absence of thrombocytosis and immune abnormalities in a patient with a PAN-like presentation [65]. Diagnosis depends on measuring serum ADA2 activity and carrying out genetic studies for *ADA2* (formerly called *CECR1*). Serum ADA2 activity was low in all patients with the deficiency, while they are normal in healthy children and in classic PAN without mutation in *ADA2* [65].

Pathology: Pathologic features are related to the vasculopathy or vasculitis. The skin and central nervous system, but also gastrointestinal system, liver, renal, and coronary tissues are affected by the vasculopathy of small- and medium-sized arteries. Skin biopsy of livedoid rash shows infiltration with polymorphonuclear leukocytes and macrophages and perivascular T lymphocytes. There was no evidence of vasculitis seen in the biopsy materials. Skin biopsy of patients with cutaneous PAN revealed non-granulomatous, necrotizing arteritis of small- or medium-sized muscular arteries, histopathologically almost similar to classic PAN (Fig. 7).

Vasculopathy may also affect the liver, kidney, and other organs. Renal artery aneurysms and/or stenosis, lymphocytic infiltration, and glomerulosclerosis are reported. Biopsy from gastrointestinal lesions shows features of inflammatory bowel disease. Histopathologic examination of liver biopsies reveals nodular regenerative hyperplasia and/or periportal sclerosis. Intestinal necrosis, bowel perforation, stenosis, or aneurysms of the mesenteric or celiac arteries are also reported [65–67].

Treatment and Prognosis: Patients with deficiency of ADA2 respond poorly to conventional treatment for PAN. However, they show excellent

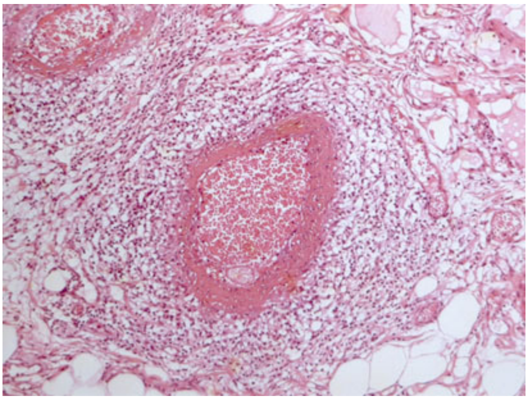

Fig. 7 Skin biopsy of a DADA2 patient showing similar microscopic appearance with PAN

response to anti-TNF drugs, in terms of successful control of fever, vasculopathy, and preventing cerebrovascular accidents [67, 69]. Patients with severe hematological involvement do not respond; hematopoietic stem cell transplant rescues hematological and immunological abnormalities [68].

Takayasu Arteritis

Takayasu arteritis is defined as a granulomatous inflammation of the aorta and its main branches [1, 2]. Although not confirmed, children seem to have a higher incidence of renal artery stenosis. This led to the inclusion of hypertension as a criterion to the new childhood criteria. According to the revised Ankara 2008 classification, *angiographic abnormalities* (conventional, CT or MR angiography of the aorta or its major branches and pulmonary arteries showing aneurysm/dilatation, narrowing, occlusion or thickened arterial wall (**mandatory**) plus one of five classifies the patient as having Takayasu arteritis [6]:

- Pulse deficit or claudication
- Four limb blood pressure discrepancy >10 mm Hg
- Bruits
- Hypertension (>95th percentile for age, sex and height)
- Elevated acute phase reactants

The etiology and pathogenesis are unknown, and the above features may include patients with renovascular hypertension due to fibromuscular dysplasia.

Clinical and laboratory features: General symptoms may occur. End-organ ischemic features ensue according to the involved vessel site. Relevant symptoms may be claudication, abdominal pain, headache, syncope, stroke, and renal hypertension due to renal artery involvement [2, 6, 70, 71]. Hypertension is the most common renal manifestation. Associated glomerulopathies have also been reported [72]. In adults, the disease is divided into pre-pulseless phase and a pulseless phase. The pre-pulseless disease is not always evident in children. Aorta and the large arteries on both sides of the diaphragm may be involved. Symptoms depend on the anatomical location of the artery and on the type of lesion (stenotic, occlusive, or aneurysmal) [6, 70].

Laboratory findings include high ESR and CRP [6]. Autoantibodies to nuclear antigens and ANCA are negative. The diagnosis depends on imaging techniques that demonstrate stenosis, and/or aneurysmal changes and/or arteritis involving the aorta and its branches. MR or CT angiography may provide evidence of Takayasu arteritis. These techniques provide information regarding thickening of the vessel wall that reflects inflammation [70]. Recent reports highlight the value of PET scan for the diagnosis. However, this scan is expensive and should be reserved for the rare instances where differential diagnoses of fibromuscular dysplasia pose a problem.

Pathology: Takayasu arteritis is a pan-arteritis, with initial inflammation being around vasa vasora and the medio-adventitial junction. A giant cell granulomatous reaction and laminar necrosis may be seen. Fragmentation of elastic fibers is prominent. Severe inflammation may lead to medial weakening, vascular dilatation, and aneurysm formation and reactive fibrosis. In the chronic phase, there is patchy mononuclear inflammatory infiltrate with medial scarring and vascularization (Fig. 8). Glomerulopathy is a rare complication [73–75].

Treatment and prognosis: Recently adult rheumatologists have published recommendations for

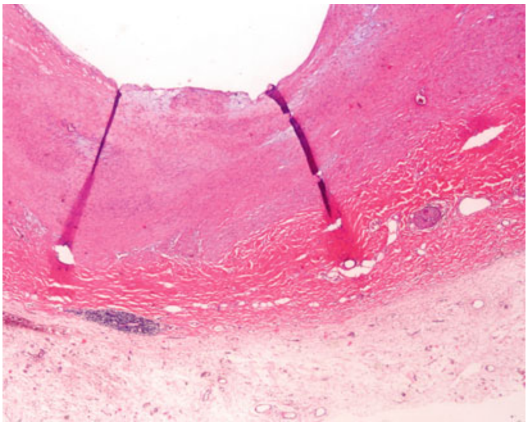

Fig. 8 TA. Chronic phase in thoracic aorta showing fibrosis in intima and fibrous thickening of the media

the treatment and management of large vessel vasculitis and Takayasu arteritis [76]. While there is lack of evidence-based data treatment in children, corticosteroids constitute the main treatment. We had suggested the use of cyclophosphamide for patients with life-threatening, severe involvement on both sides of the diaphragm, and methotrexate for limited disease [77].

Recently therapy with anti-TNF agents and tocilizumab has been reported to be effective in achieving clinical response in patients with Takayasu arteritis [78, 79].

For patients who require revascularization, both surgical and endovascular procedures can be performed, with low morbidity and mortality. The prognosis has improved with lower relapse rates. However, the evaluation of disease activity is a major problem in the management and follow-up. Any sign of vascular ischemia, rising ESR, and new angiographic abnormalities should suggest a relapse of the disease that requires appropriate management.

Behçet Disease

Behçet disease is classified according to the criteria used for adults where the presence of oral ulceration is mandatory. Two other criteria are required: genital ulceration, skin findings, eye involvement, and a positive pathergy test [80]. According to pediatric criteria, three of the following six are required to classify a patient as Behçet disease: oral aphthosis, genital ulceration, skin features, eye involvement, CNS involvement, and vascular involvement (venous thrombosis, arterial thrombosis, or arterial aneurysms) [81]. Behçet disease is a vasculitis that affects the mucosal membranes and the skin, just as Kawasaki disease. Epidemiology suggests spread through the ancient “silk road.” The disease is characterized by periods of exacerbations and remissions.

Behçet disease is rare in childhood with certain characteristics of interest. Behçet disease is the only vasculitis that affects vessels of all sizes and both arteries and veins, except systemic lupus erythematosus [1]. Behçet disease has a wide range of renal involvement, albeit rare [82]. Common glomerular lesions include crescentic GN, proliferative GN, and immunoglobulin A (IgA) nephropathy [82]. Aneurysms may be located throughout the renal artery, from the orifice of the main artery to intrarenal microaneurysms.

Pathology: Behçet disease has no strict pathognomonic features on histopathology, and wide spectrum of microscopic changes. Skin lesions show leukocytoclastic vasculitis and panniculitis with superficial and deep perivascular lymphocytes and neutrophils (Fig. 9) [80, 83]. Treatment depends on the organ system involved and frequently requires corticosteroids and immunosuppressive treatment. Adult rheumatologists have suggested recommendations for the treatment of BD [84].

In conclusion, kidney is an organ that is frequently involved in the vasculitides. It has a major impact on the morbidity of the disease. Careful assessment and effective management of these patients are essential. We await controlled, long-term studies to shape our evidence-based treatment in these children. We now have the classification criteria and the outcome assessments to guide us in our study designs. The Pediatric Vasculitis Assessment Score has been developed and validated for the use by pediatricians to assess treatment outcomes and evaluate the activity of disease in their patients. The pediatric community is ready for collaborative studies in childhood vasculitides.

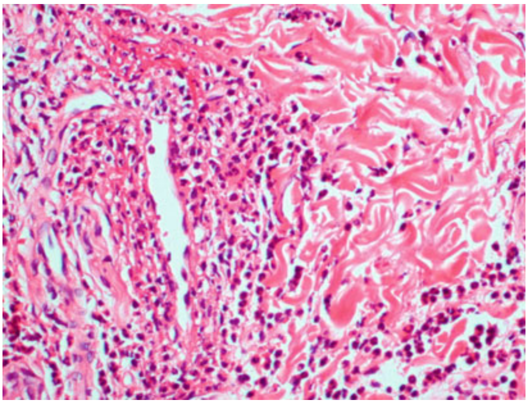

Fig. 9 Behçet's disease. Edema of the endothelial cells, fibrinoid degeneration, neutrophilic infiltration, and leukocytoclasis (destroyed leukocytes as nuclear dust) in cutaneous small vessel walls

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Lupus Nephritis

20

Scott Wenderfer, Sherene Mason, Christine Bernal, and
Clovis Artur Almeida da Silva

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Abstract

Childhood-onset systemic lupus erythematosus (cSLE) is a subset of SLE with onset prior to 16–18 years of age. Around 15% of patients with SLE present in childhood; patients with cSLE have a greater genetic component to their disease, more systemic involvement, and more severe disease course including higher risk for developing nephritis. Patients with cSLE develop loss of tolerance to self at an earlier time than patients with adult-onset SLE, or loss of tolerance progresses sooner to autoantibody production, immune complex deposition, and immune-mediated tissue injury. We review the diagnosis, classification criteria, and management of kidney disease in cSLE, by pediatric nephrologists and rheumatologists. Although the evidence basis for management often primarily relies on adult studies, there is growing literature on cSLE around the world. Fortunately, 5- and 10-year mortality is lower than in adult-onset SLE, and patient and renal survival has improved over the years. However, relapses are common, especially during the transition from adolescence to adulthood. Patients with cSLE also accrue organ damage and co-morbid conditions from lupus and their therapies, including cardiovascular, infectious, ocular, skeletal, growth, endocrine, and reproductive complications.

Keywords

Systemic lupus erythematosus · Lupus nephritis · Nephrotic syndrome · Autoantibodies · Complement · Biopsy

Synonyms

BAFF (B-cell activating factor); BLyS (B-lymphocyte stimulator); Calcineurin inhibitors; Chronic kidney disease stage 5; Cyclophosphamide; End stage kidney disease;

Mycophenolate mofetil; Plasma exchange; Plasmapheresis;

Introduction

Systemic lupus erythematosus (SLE) is a complex chronic autoimmune disease with multisystemic involvement characterized by the production of antibodies against self-antigens, immune complex formation, and immune dysregulation. Childhood-onset SLE (cSLE) accounts for 10–20% of SLE cases with an incidence of 0.3–0.9 per 100,000 and a prevalence of 3.3–24 per 100,000 [72]. Comparative studies have shown differences between cSLE and adult-onset SLE (aSLE). In a meta-analysis, cSLE was associated with severe clinical features particularly neurological involvement, renal involvement, oral ulcers, malar rash, vasculitis, fever, ocular, and hematological manifestations [27]. Children and adolescents with SLE have fulminant disease onset and course than adults with SLE, resulting in two to three times higher mortality [102].

cSLE has a strong female preponderance, with a female-to-male ratio 4:3, increasing to 9:1 during the reproductive years [102]. There is a difference in the ethnic composition of SLE in the world with higher frequency seen among African Americans, Asians, Hispanics, and Native Americans. In adults, non-Caucasian ethnicity has been identified as a risk factor for poor outcome [44] but in a study on cSLE, disease activity and damage were strongly associated with major organ disease independent of the patient's ethnicity. Non-Caucasians were significantly younger and more likely to have lupus nephritis [61].

The clinical phenotypes of SLE are heterogeneous; hence, manifestations are protean and variable. Classification criteria, established to identify a well-defined patient population suitable for research, are helpful in clinical practice. The classification of SLE, endorsed by the American College of Rheumatology (ACR) in 1982 [154] and revised later [63], has been most commonly applied. Due to low sensitivity in detecting early SLE, alternative classifications have been developed by the SLICC (Systemic Lupus International Collaborating Clinics) [120] and EULAR/ACR [6]. EULAR/ACR criteria total score $\geq$ 13, as opposed to the initially proposed score $\geq$ 10, most appropriately classifies cSLE [127]. In 2020, a kidney biopsy with lupus nephritis class III or IV and presence of a positive antinuclear antibodies (ANA) are deemed sufficient to classify a patient as SLE (Table 1).

Pathogenesis

Animal Models

The complexity and heterogeneity of the clinical presentation in SLE, combined to the inherent limitations of clinical research, have made it necessary to study various rodent models to dissect the cellular, genetic, and metabolic mechanisms of disease [90]. The major spontaneous models include the MRL/lpr, BXSB/Yaa, and (NZB x NZW)F1 models. In addition, lupus-like disease has been induced in rodents using pristane, nephrotoxic serum, and passive immunization with patient sera. MRL mice, carrying a pathologic variant named *lpr* in the FAS gene locus, develop lymphoproliferation due to a defect in apoptosis that ultimately leads to autoimmunity. Disease in the BXSB mouse strain is attributable to the Y-autoimmune accelerator (Yaa) locus, which duplicates 16 genes, including TLR7 (Toll-like receptor 7), an innate immune sensor encoded on the X chromosome that binds single-stranded RNA and activates type I interferon signaling. New Zealand white and black mice carry numerous gene variants predisposing to autoimmunity, but only F1 progeny develop tissue injury and immune complex glomerulonephritis (GN). Nephrotoxic serum (▶ Chap. 21, "Autoantibodies and Kidney Diseases") is generated in sheep or rabbits after immunization with purified mouse glomerular basement membrane (GBM). Injection of this serum into mice leads to inflammatory GN due to Fc-receptor-mediated recruitment of phagocytes binding immune complexes bound to GBM. These models have contributed major advances to the field, including numerous potential targets for pharmaceutical development. Each model shares specific subsets of attributes with SLE, and investigators evaluating novel agents need to select the animal model carefully [90].

Table 1 Classification criteria for systemic lupus erythematosus [6, 63, 120]

	ACR 1997 [63]	SLICC 2012 [120]	ACR/EULAR 2019 [6]	
Requirement	4 out of 11 criteria	4 criteria, ≥ 1 clinical and ≥ 1 immunologic criterion, *or* biopsy proven nephritis and ANA positive	ANA at least 1:80; plus a score of ≥ 10 points	
Clinical				
				Points
Mucocutaneous	1. Malar rash 2. Photosensitivity 3. Discoid rash 4. Oral, nasopharyngeal ulcers	1. Acute cutaneous lupus 2. Chronic cutaneous lupus 3. Oral or nasal ulcers 4. Non scarring alopecia	Acute cutaneous lupus Subacute cutaneous lupus or discoid lupus Oral ulcers Non scarring alopecia	6 4 2 2
Joints	5. Non-erosive arthritis	5. Synovitis	Synovitis	6
Serositis	6. Pleuritis *or* pericarditis	6. Pleurisy, pleural effusion, pleural rub, pericardial pain, pericardial rub, *or* pericarditis	Pleural *or* pericardial effusion	5
			Acute pericarditis	6
Renal	7. Persistent proteinuria (>0.5 g/24 h, *or* $>$ 3+ dipstick); cellular casts	7. Random proteinuria (>0.5 mg/mg), 24 h proteinuria (>0.5 g/24 h), *or* red blood cell casts	Proteinuria (>0.5 g/24 h)	4
			Lupus nephritis II *or* V	8
			Lupus nephritis III *or* IV	10
Neurologic	8. Seizures *or* psychosis	8. Seizures *or* psychosis; mononeuritis, myelitis, neuropathy, *or* confusional state	Seizure	5
			Psychosis	3
			Delirium	3
Hematologic	9. Hemolytic anemia, leukopenia ($<4000/\text{mm}^3$), lymphopenia ($<1500/\text{mm}^3$), *or* thrombocytopenia ($<100{,}000/\text{mm}^3$) on 2 occasions	9. Hemolytic anemia 10. Leukopenia ($<4000/\text{mm}^3$) 11. Lymphopenia ($<1000/\text{mm}^3$) 12. Thrombocytopenia ($<100{,}000/\text{mm}^3$)	Autoimmune hemolysis Leukopenia Thrombocytopenia	4 3 4
Constitutional			Fever	2
Immunologic				
	10. Anti-dsDNA, anti-Smith antiphospholipid antibody	1. Anti-dsDNA	Anti-dsDNA	6
	11. Positive ANA	2. Anti-Smith	Anti-Smith	6
		3. Antiphospholipid antibody	Antiphospholipid antibody	2
		4. Low complement (C3, C4, CH_{50})	Low C3, *or* low C4	3
		5. Direct Coombs test	Low C3 *and* low C4	4
		6. Positive ANA		

*abbreviations: *ANA* antinuclear antibody; *dsDNA* double stranded *DNA*; CH50 total hemolytic complement

Genetics

Single gene defects or Mendelian-inherited associated with cSLE is very rare [37] (Table 2). Some mutations may affect several immune pathogenetic pathways: complement system, phagocytes oxidase system, apoptosis, nucleic acid repair, DNA degradation, DNA sensing, type I interferon, and B-cell development [37]. A variety of single mutations are associated with susceptibility to develop lupus nephritis.

The most frequent monogenic lupus is homozygous deficiency of early components of the classical complement pathway (C1q, C1r/s, C4, and C2) [70]. SLE or lupus-like manifestations have been detected in 81–90% of homozygous C1q deficient, 75% of homozygous C4-deficient, 65% of homozygous C1r/C1s deficient, and 10% of homozygous C2-deficient subjects [37]. Lupus nephritis has been described in 17–90% of these patients [37]. Complement deficiencies seem to predispose to SLE and lupus-like syndrome due to tolerance abnormalities, aberrant apoptotic bodies clearance and poor clearance of immune complexes, perpetuating and intensifying the immune response. Non-cleared debris would operate as antigens to stimulate helper T cells and production of multiple autoantibodies [142].

A set of genes associated with monogenic lupus showed systemic autoimmunity and was associated with lupus phenotype. Lupus nephritis was reported in 30–100%, mainly with mutations in the following: *PRKCD, C1QA, C1QB, C1QC, C1R, C1S*, and *C4* [37]. Genetic analysis (whole exome sequencing) should be indicated for SLE or lupus-like patients with early onset of clinical features (<6 year), parental consanguinity, family history for autoimmune condition in a first-degree relative, and severe disease with tissue scaring [70].

Environment

Epidemiologic evidence exists for association of silica, Epstein-Barr infection, and cigarette smoking with SLE [15]. Alcohol consumption is associated with decreased SLE risk. There are conflicting data associating infections, vaccinations, air pollution, ultraviolet (UV) light, solvents, pesticides, and heavy metals, for example mercury. The hydrocarbon pristane, a component of mineral oil, is used to model lupus in mice [90]. While UV radiation exposure is known to exacerbate preexisting lupus, it is unclear whether this is a risk factor for its development. Biologic mechanisms linking environmental exposures and SLE include oxidative stress, systemic inflammation, cytokine upregulation, and molecular mimicry to viral antigens. Alterations in the gut microbiome include impaired gut-barrier function, expansion of *Proteobacteria*, reduction of *Firmicutes* spp., and inverse association between disease activity and diversity of microbiota [143]. Development of antibodies against *Ruminococcus gnavus* correlate with double-stranded (ds) DNA antibodies and progression to lupus nephritis.

The environment has been proposed to interact at the epigenetic level in lupus [13]. UV exposure is associated with reduced DNA methyltransferase 1 expression in patients with SLE. Dietary content of transmethylation micronutrients (betaine, methionine, and choline as methyl donors; zinc, folate, and B-vitamins as methyl cofactors) correspond with the severity of lupus in rodent models. Flares in lupus may be linked to epigenetic disequilibrium. While there is decreased DNA methylation of CpG islands at type-1 interferon genes and other susceptibility loci in active lupus, there is increased methylation of genes required for regulatory T-cell function.

Hormonal Factors

Strong epidemiologic evidence supports associations of oral contraceptives, early age at menarche, postmenopausal hormone therapy, and endometriosis with increased risk of SLE [15]. Female sex is a strongly predisposing factor, and the overwhelming majority of cases occur among women during

Table 2 Monogenic conditions associated with systemic lupus erythematosus (SLE)

Disease	Gene mutated	Inheritance	Clinical features of lupus	Non-SLE features
Aicardi Goutières syndrome	*TREX1*	AD or AR*	Early-onset; chilblain lupus	Encephalopathy, intracerebral calcification
	RNASAH2A/B/C	AD		
	ADAR1	AD		
	SAMHD1	AR		
	IFIH1	AD		
STING (stimulator of interferon genes deficiency)	*TMEM173*	AD	Chilblain lupus; interstitial lung disease	Vasculopathy with onset in infancy
SPENCDI (spondyloenchondrodysplasia with immune dysregulation)	*ACP5*	AR	Early-onset (frequent LN, cytopenia)	Osseous dysplasia short stature
DNase 1 deficiency	*DNASE1*	AD	Adolescent onset, dsDNA(+)	–
DNase 1 IL3 deficiency	*DNASE1L3*	AR	Early-onset illness, nephritis, ANCA(+)	Hypocomplementemic urticarial vasculitis
PKCδ deficiency	*PRKCD*	AR	Early-onset	Lymphoproliferation
C1q deficiency	*C1QA/B/C*	AR	Early-onset; nephritis, CNS, and photosensitivity common	Susceptibility to encapsulated bacterial infections
C1r/C1s deficiency	*C1R/C1S*	AR	Early-onset, nephritis frequent	
C2 deficiency	*C2*	AR	Photosensitivity and arthritis; nephritis: mild or absent	
C3 deficiency	*C3*	AR	Malar rash, photosensitivity, arthralgia, Raynaud	
C4 deficiency	*C4A/B*	AR	Organ involvement, nephritis	
Chronic granulomatosis Disease	*CYBB*	X-linked	Discoid lupus	Susceptibility to pyogenic, fungal infections
	NCF1/2	AR		

*abbreviations: *AD* autosomal dominant, *AR* autosomal recessive

childbearing years. Both sex hormones and X chromosome factors are thought to be responsible. Individuals with Klinefelter syndrome (47,XXY) also have increased incidence of SLE. Epigenetic modification of genes on the inactive X chromosome has been proposed as a possible mechanism [132]. In a meta-analysis, significantly lower testosterone and dehydroepiandrosterone, and higher estradiol and prolactin are seen in women with SLE [99]. Data on menstrual irregularity, breastfeeding duration, number of ovulatory years, and parity have been inconsistent.

Immune Complexes (Antibody-Mediated Immunity)

SLE is characterized by loss of tolerance (▶ Chap. 21, "Autoantibodies and Kidney Diseases") to numerous self-antigens, although no one unifying event has been identified. Many patients show defective clearance of cell debris, which triggers autoimmune responses against large intracellular antigens (chromatin, nucleosomes, DNA, RNA, and ribonuclear proteins) that would otherwise evade immune responses.

Others demonstrate increased circulating levels of extracellular vesicles, which are membrane-bound microparticles, exosomes, or apoptotic bodies containing phospholipids and nuclear or mitochondrial debris. Defects in the classical pathway of complement (such as C1q), DNase enzymes, and phagocyte receptors (encoded by *FCGR2B*, *FCGR3*, *ITGAM*) contribute to an increased exposure to extracellular vesicles [37].

In SLE, although autoantibodies can be detected against nearly any portion of the human proteome, the most common antibodies detected are antinuclear antibodies (ANA). However, individuals can test positive for ANA without developing manifestations of SLE. Numerous assays can be used to profile ANA reactivity; specificity for anti-double stranded DNA or Smith antigen is more in patients with SLE.

Immune complexes form when antibodies bind to their specific antigen. Circulating immune complexes form against antigens in the blood or extracellular fluid, and can deposit in vascular capillary beds anywhere in the body. In situ immune complex formation occurs when autoantibodies bind to their antigen on cell surfaces or extracellular matrix. In situ immune complexes can also form against circulating antigens that are trapped in necrotic tissue, basement membranes, or cellular glycocalyces. Immune complexes can also become quite large and deposit on tissues. Fixed deposits are immune deposits that remain visible after tissue processing for viewing by electron microscopy. Although immune complex formation can be helpful in opsonization and clearance of foreign or toxic substances, abundant immune complexes can lead to inflammation by leukocytes via binding to Fc-receptors [121].

Innate Immunity

Patients with cSLE have increased numbers of the low-density granulocyte subset of neutrophils [101]. The production of neutrophil extracellular traps (NETs) has been shown to be important in the pathogenesis [94]. NETosis in response to immune complex binding to neutrophils can propagate immune injury and further expose nuclear antigens to circulating autoantibodies.

The type-I interferon pathway has also proven to be pathogenic in many patients with SLE. Administration of recombinant human IFN to treat viral infections and malignancies has resulted in de novo cases of lupus, and disease improved with cessation of therapy [111]. The disease genes TLR7 and TLR9 encode intracellular receptors for nucleic acids. TLR activation leads to production of type-I interferon as part of the innate immune response. Polymorphisms of *IRF5*, *IKZF1*, and *STAT4* are all linked to development of immune complex GN and their encoded proteins all mediate type-I interferon signaling in inflammatory cells as well as resident kidney cells [39]. Defects in other disease genes that regulate another innate immune response, the nuclear factor kappa-B pathway (*TNFAIP3*, *TNIP3*), also predispose to development of autoimmune nephritis. Hypocomplementemia is due to consumption of complement proteins, mostly due to classical pathway activation (▶ Chap. 22, "Membranoproliferative Glomerulonephritis and C3 Glomerulopathy in Children") by immune complexes.

Lymphocytes (Cell-Mediated Immunity)

The loss of tolerance to self-antigens in SLE can also be caused by abnormalities in lymphocytes. The risk variant for *PTPN22* produces a mutant signaling protein that leads to failure to eliminate autoreactive B cells [100]. Reduced expression of BLK, another B-cell signaling molecule, promotes nephritogenic lymphocyte generation in mice prone to lupus [136]. BAFF (B-cell activating factor) promotes the humoral immune responses that then sustain autoantibody production [19].

In addition to providing T cell help to autoreactive B cells, tissue injury in SLE can occur by direct T-cell-mediated cytotoxicity [38]. Follicular helper CD4 cells drive the formation of antibody secreting cells. Type 17 T-helper cells produce IL-17 and promote inflammation and humoral immunity. Cytotoxic CD8+ T cells release granzymes and perforin to lyse cellular targets. Unique to patients with SLE, double negative (CD3+, CD4–, CD8–) T cells escape negative selection and promote inflammation. Regulatory T cells are produced to suppress effector T-cell responses, but can become dysregulated in SLE [38].

Pathogenesis of Nephritis

Tissue damage in multiple organs requires more than just autoantibody generation. Experimentally, anti-dsDNA and anti-nucleosome antibodies can deposit in the kidney and cause proteinuria [82]. Besides ANA, autoantibodies against alpha-actinin, alpha-enolase, annexin A1, and vimentin in the kidney associate with GN. Anti-C1q antibodies occur at higher titers in patients with LN and co-localize with complement deposits in their glomeruli [157]. However, experimentally and clinically, immune complex deposition in the kidney can be detected in the absence of histopathology or clinical disease.

It is not clear why only certain children with SLE develop nephritis. CC-chemokine ligand 2/monocyte chemoattractant protein-1 (CCL2/MCP-1) is involved early in the GN process [4], and tumor necrosis factor is involved at onset of proteinuria. Type-I interferons cause direct injury to cells in the glomerulus. Genetic polymorphisms in the *KLK* locus with decreased renal concentrations of kallikrein also associate with the development of nephritis. Numerous inflammatory cells play a role in pathogenesis of nephritis [4]. Neutrophil and endothelial cell microparticles contribute to immune complex formation and localize to glomerular immune deposits. Resident and infiltrating macrophages and dendritic cells participate in inflammation and development of kidney fibrosis.

Cellular infiltrates in lupus nephritis are common and are composed of phagocytes and B and T lymphocytes [30]. T-cell subsets, including cytotoxic CD8 cells, type 17 T-helper CD4 (TH17) cells, follicular helper CD4 (TFH) cells, and double negative TH cells, have all been implicated. Effector T cells, together with numerous soluble mediators, elicit chronic inflammation within the glomeruli and tubulointerstitium.

Immune complexes may bind directly to glomerular mesangial [137] and endothelial cells [152]. In mesangial cells, binding to Fc-receptors causes complex internalization, cellular proliferation, and release of inflammatory cytokines [121, 152]. Moreover, mesangial cells produce more extracellular matrix after treatment with anti-dsDNA antibodies. Anti-dsDNA antibodies also bind to annexin II on mesangial cells with functional responses [172].

Clinical Manifestations

SLE can present with intermittent or persistent symptoms, single or multiple organ involvement, and most often with constitutional symptoms of fever (51%), malaise, anorexia, and weight loss. Main clinical features are mucocutaneous involvement (80–96%), arthritis (82%), kidney manifestations (50%), and hematologic features (43–46%), with an unpredictable natural history. In general, children with SLE have more severe disease than their adult counterparts, with more organ involvement, more laboratory abnormalities, and requiring frequent use of immunosuppressive agents [150].

Kidney Involvement

Approximately half of children with SLE develop kidney involvement at one point in their disease course. Frothy or tea-colored urine, edema, anasarca, and hypertension, which may or may not be associated with increased creatinine levels, are the most common manifestations of childhood LN. Eighty to 90% of childhood lupus nephritis develops within the first year of diagnosis and is a significant cause of morbidity and mortality across all ages. Between 10 and 30% progress to kidney failure within 15 years [125].

Disease manifestations and outcomes particularly are heterogeneous. The American College of Rheumatology (ACR) 1997 revised classification criteria for SLE described kidney involvement as persistent proteinuria >0.5 g per day, or greater than 3+ if quantitation is not performed, or presence of granular casts which may be red blood cell, hemoglobin, tubular, or granular casts, or mixed. A similar criterion is stated by the SLICC classification criteria, urine-protein-to-creatinine ratio, or urine protein representing 500 mg protein/24 h, and histopathologic findings of lupus nephritis [58]. The 2019 European League Against Rheumatism (EULAR)/ACR criteria for SLE defines proteinuria as above [6], and follows the lupus nephritis classification of the 2003 criteria of the International Society of Nephrology/Renal Pathology Society (ISN/RPS).

Urine sediment analysis helps distinguish proliferative from non-proliferative forms of kidney involvement. When >50% of affected glomeruli have hypercellularity, necrosis, or large (hyaline) deposits, the clinical symptoms are more severe, with active urine sediment, nephrotic syndrome, hypertension, and AKI/CKD. Other forms of renal involvement include tubulointerstitial nephritis, thrombotic microangiopathy (TMA; Renal Involvement in Children with Hemolytic Uremic Syndrome (HUS) and Thrombotic tHROMBOCYTOPENIC Purpura (TTP)), noninflammatory necrotizing lupus vasculopathy, and lupus vasculitis (▶ Chap. 19, “Renal Vasculitis”).

Extrarenal Manifestations

Mucocutaneous

Skin manifestations of lupus are common and may be the first sign of disease. The lesions can be classified as acute, subacute, or chronic. The butterfly or malar rash is an acute violaceous or pink, non-scarring, photosensitive rash crossing the nasal bridge and sparing the nasolabial folds. Subacute lesions are annular violaceous to red scaly plaques that can erupt on extremities and are non-scarring as well. Rashes that are chronic are scarring and usually involve the scalp, V-region of the neck, and face. The other chronic lesions of lupus include panniculitis and chilblains.

Nonspecific cutaneous symptoms of lupus can be related to vasculitis and vasculopathy associated to lupus. Purpura and urticarial vasculitis are examples of the former, while livedo reticularis, thrombophlebitis, and Raynaud phenomenon represent the latter. Alopecia of lupus is diffuse, non-scarring hair loss, although it can also be patchy. Single or multiple ulcerations of the oral, gingival, and nasal mucosa characterize active disease [7].

Musculoskeletal

Joint and muscle symptoms of lupus are present in approximately 50% of patients. Arthralgia is a common complaint. Oligo- or polyarthritis associated with morning stiffness is non-deforming and involves both the large and small joints. Tendinitis and tenosynovitis can be painful and disabling. Approximately 5% patients with musculoskeletal involvement have myositis [135].

Nervous System

Neuropsychiatric lupus is more frequent in cSLE than in adult onset SLE. It can affect the central, peripheral, and autonomic nervous systems and can result in headaches, acute confusional states, seizures, psychosis, cognitive deficits/dysfunction, chorea, cerebrovascular accident, cranial nerve palsies, mood disorder/depression, movement disorder, aseptic meningitis, demyelinating syndrome, myelopathy, dysautonomia, and pseudotumor cerebri. Headache is the chief symptom (80–90%), and may be sometimes accompanied by seizures [146].

Cardiovascular

Similar to neuropsychiatric symptoms, cardiovascular manifestations are more common and more severe in children than in adults. These include chest pain, pericardial effusion, valvular disease pericarditis, and cardiac failure; the most frequent vascular manifestations are vasculitis and thromboembolism. SLE is independently associated with a higher likelihood of having pericarditis or myocarditis. SLE may present with Libman-Sacks endocarditis, an aseptic endocarditis commonly affecting the mitral valve. It is rare and is best evaluated by trans-esophageal echocardiogram [80]. Acute cardiac and vascular manifestations of SLE are associated with significant mortality; however, chronic cardiovascular effects of SLE are a burden as children survive into adulthood. About 36% of the cardiac complications of SLE are silent [57].

Pulmonary

Pulmonary involvement occurs in 40% of cSLE within the first year of diagnosis and doubles at any point during the disease course. Cough is a common complaint; recurrent or persistent coughing is an indication of several respiratory disorders or complications related to lupus, with pleuritis, with or without unilateral or bilateral effusion, being the most common [124]. Acute lupus pneumonitis and alveolar hemorrhage are rare entities, and associated with grave outcomes. Dyspnea, hypoxemia, and falling hematocrit heralds diffuse alveolar hemorrhage [144].

Asymptomatic or subclinical pulmonary abnormalities are present in a significant number of cSLE patients through spirometry or DLCO, including airway defects in 50%. Pulmonary hypertension and interstitial lung disease, the latter presenting with exertional dyspnea and pleuritic pain, are among the chronic pulmonary complications of cSLE. Pulmonary hypertension can be associated with signs of right heart failure [124].

Gastrointestinal

The most frequent gastrointestinal symptom in cSLE is abdominal pain, followed by diarrhea, and jaundice. Findings of ascites, peritonitis, gastritis, enteritis, colitis, and hepatomegaly are also documented [148]. Transaminitis may occur due to lupus activity, or a consequence of treatment. Autoimmune hepatitis associated with lupus is a progressive inflammatory liver disease and can coexist with cirrhosis and cholangitis. Diagnosis of lupus enteritis, a rare disease entity, is made on computed tomography findings of dilated bowels and bowel wall thickening [148].

Hematologic and Reticuloendothelial System

SLE can affect at least one hematopoietic lineage, leading to anemia, leukopenia, lymphopenia, and thrombocytopenia [135]. The decrease in hemoglobin can be attributed to anemia of chronic disease, iron deficiency, or hemolysis. Autoimmune hemolytic anemia, defined as hemoglobin <10 g/dL and reticulocytosis or a positive Coombs test, is more common, severe, and likely to be associated with hepatosplenomegaly in cSLE than in adult-onset SLE. Leukopenia and thrombocytopenia are common as well and usually mild. While there is no evidence of a significant association between overall reduction of white blood cell count and infection, lymphopenia and neutropenia are strongly associated with the risk of major infections. Thrombocytopenia, similar to anemia, is chiefly autoimmune in nature, and may be related to antiphospholipid syndrome (aPLS) [68].

Splenomegaly and lymphadenopathy may be seen in active disease; prolonged duration of disease may result in splenic atrophy in cSLE. Kikuchi-Fujimoto disease, or histiocytic necrotizing lymphadenitis, is a rare disorder outstandingly associated with SLE [149].

Endocrine

Patients with cSLE can exhibit hypothyroidism or hyperthyroidism. Hashimoto thyroiditis is the most common autoimmune thyroid disease at any age, most frequently age at adolescence. It often manifests as hypothyroidism, is linked to thyroid peroxidase and thyroglobulin antibodies, and is associated with connective tissue diseases [91]. Hyperglycemia is usually transient; however, diabetes mellitus type 2 may develop with long-term high dose corticosteroid treatment [59]. Diabetes type 1, on the other hand, may precede or follow lupus diagnosis as part of polyautoimmunity, as in Hashimoto thyroiditis and autoimmune hepatitis [140]. Adrenal insufficiency may occur with aPLS, unusual stress, or abrupt corticosteroid withdrawal [83].

Ocular

Eye irritation or itchiness due to dryness is common (40%). Gradual or sudden blurring of vision is of concern, and although causes such as retinal vein or artery occlusion and uveitis (5%) are rare, the outcomes may be serious. Findings in lupus retinopathy include cotton wools spots, intraretinal hemorrhages, and vascular tortuosity

[47]. Patients with cSLE should be monitored for medication-related damage, particularly retinal toxicity and cataracts secondary to long-term intake of hydroxychloroquine and steroids, respectively [59].

Drug-Induced Lupus

Approximately 10% of SLE cases are drug-induced lupus [169]. This spectrum includes clinical features and laboratory abnormalities of SLE with persistent drug exposure, and generally lacking of severe SLE complications [160]. The most frequent medications associated with lupus include antiarrhythmic (procainamide, quinidine), antihypertensive (hydralazine), antimicrobial (minocycline), anticonvulsant (carbamazepine, phenytoin), and TNFα blockers (infliximab, adalimumab, etanercept) [160]. The highest risks of drug-induced lupus are associated with use of procainamide (15–20%) and hydralazine (8–13%).

Clinical features of lupus occur several weeks, months to years after drug exposure, and improve after discontinuation of the putative drug. The clinical involvement of drug-induced lupus includes mainly mild to moderate disease features, such as fever, fatigue, myalgia, arthralgia, arthritis, serositis, sicca syndrome, and Raynaud phenomenon [160]. The most frequent drug-induced skin manifestation is subacute cutaneous lupus, with rare cases reported of chronic lupus, as discoid lupus. Lupus nephritis, neuropsychiatric, hematologic abnormalities, and hypocomplementemia are rarely described [160].

ANA have been observed in 90–100% of patients with drug-induced lupus [160], particularly with homogeneous immune-staining. Anti-histone antibodies are characteristic of drug-induced lupus, identified in 90–95% of patients [160], specifically directing the (H2A-H2B)-DNA complex. Anti-dsDNA and anti-Sm antibodies are rarely observed in drug-induced lupus.

Neonatal Lupus

Neonatal lupus arises from the passive placental transfer of pathogenic maternal autoantibodies to SSA (Ro) or SSB (La). It occurs in up to 2% of offspring to mothers with these antibodies irrespective of maternal history of autoimmune disease. Clinical manifestations include cardiac, cutaneous, hematologic and hepatic, with cardiac complications being the most serious. Conduction system abnormalities including congenital heart block is estimated to occur in 1 in 15,000 to 1 in 22,000 live births independent of any structural abnormalities of the heart [28]. The risk of cardiac neonatal lupus in subsequent pregnancies in mothers with a previously affected child is 17% [93]. Congenital heart block portends a significant mortality in 20 to 30% of neonates, with 67% of affected survivors requiring pacemaker implantation.

The rash of cutaneous lupus is located primarily on the scalp and periorbital area, and is induced and exacerbated by exposure to ultraviolet light. It spontaneously resolves as maternal antibodies are cleared from the neonatal circulation by 6–8 months of age. Hematologic findings include anemia, thrombocytopenia, and neutropenia. Hepatobiliary manifestations occur in 10–25% of patients and include elevation in transaminases, cholestasis, or liver failure. In a series of 50 infants born to mothers with anti-SSA/Ro antibodies who themselves were positive for the same antibodies, gradual decrease of antibodies occurred simultaneously with disappearance of noncardiac features. At 9 months of life, only 10% had persistent antibodies [175].

Antiphospholipid Syndrome (aPLS)

Childhood-onset SLE has a high prevalence of antiphospholipid antibodies, resulting in high risk of thrombosis. Pediatric aPLS (▶ Chap. 21, "Autoantibodies and Kidney Diseases") is characterized by one or more clinical episodes of arterial, venous or small-vessel thrombosis, in any tissue or organ, and the presence of at least one antiphospholipid antibody: anti-cardiolipin (IgG, IgM), lupus anticoagulant, and anti-β2 glycoprotein-I (IgG, IgM) [11].

Primary aPLS occurs in patients without any signs or symptom of autoimmune diseases. Secondary aPLS is associated with autoimmune disease or other acute or chronic disease, especially cSLE and lupus like disease [11]. A recent multicenter study on 1512 cSLE patients described cumulative aPLS in 4%. Autoimmune thrombosis was diagnosed at disease onset in 58% patients

[68]. Venous and arterial thromboses are the main sites of thrombotic manifestations in cSLE-related APS [68]. Non-thrombotic manifestations associated with cSLE-APS occurred in 31%, mainly livedo reticularis in 21%, valvar thickening in 6%, and valvar vegetations not related to infections in 3% [68]. Pregnancy morbidity has been infrequently reported in adolescents (1–3%) [68]. Autoimmune thrombosis involving kidneys has been rarely reported. An international registry revealed that thrombotic microangiopathy involving the kidneys occurred in 2% of pediatric patients with aPLS at disease onset, renal vein thrombosis was observed in 1%, and renal arterial thrombosis in 1% [11].

Pediatric catastrophic antiphospholipid syndrome (CAPS) is defined as severe multiorgan thrombosis developing over a short time period, and can occur in children with SLE [18]. Kidney involvement is present in 63% of episodes of pediatric CAPS, and includes kidney failure (48%), arterial hypertension (24%), and proteinuria (13%). Approximately, 25% patients die at the moment of the catastrophic event [18].

Kidney Biopsy, Pathology

Kidney biopsy is the gold standard for the diagnosis of lupus nephritis. Practice patterns for initial and repeat kidney biopsy have been published for cSLE [166]. Most specialists use the indications for initial kidney biopsy developed by the American College of Rheumatology (Table 3) [56], whereas others use more inclusive indications.

Histological Classification

The kidney pathology (▶ Chap. 7, "Technical Aspects of Renal Pathology". Alternatively, we could include a figure with representative histopathology of all 6 classes, as in 7th edition) seen in SLE patients varies widely. As in adult onset SLE, either the World Health Organization classification, revised last in 1995 [33], or the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification, revised in 2018 [12], is used to classify the histological findings (Table 4). The prevalence for each lupus nephritis class is similar in cSLE and adult onset SLE [20]. The utility of distinguishing between histological classes, such as proliferative, and membranous lupus is supported by studies of SLE in children [173].

Although clinical presentation can predict histological findings in lupus nephritis, exceptions are common. Isolated asymptomatic hematuria and/or mild proteinuria are seen in lupus nephritis class I or II. Proliferative lupus nephritis often presents with the acute nephritic syndrome, defined by hematuria (microscopic or macroscopic), accompanied by hypertension and proteinuria (mild to nephrotic-range) (▶ Chap. 8, "Approach to the Child with Hematuria and/or Proteinuria"). In lupus nephritis class IV, symptoms are similar to class III, but with higher risk for progression to kidney failure. Class V (membranous) disease usually presents with isolated nephrotic syndrome. Some patients show presence of mixed proliferative and membranous lesions. Over time, the class of lupus nephritis can change, especially with disease flares.

Activity and Chronicity

Active lesions are amenable to immunosuppressant therapy, while chronic lesions represent nonreversible damage [12], requiring supportive therapy. Activity index and chronicity index quantify mainly glomerular injury (Table 5), and tubulointerstitial activity index quantifies extra-glomerular disease [60]. As in adults, risk factors for poor outcome in cSLE include activity index ≥ 7, chronicity index ≥ 4, and tubulointerstitial activity index >5 [24, 174]. Fibrinoid necrosis and cellular/ fibrocellular crescents are weighted as they historically associate with higher rates of long-term kidney failure. The activity and chronicity indices may not reflect the severity of lupus nephritis, and may not respond to therapy at the same rates as clinical and laboratory findings.

Table 3 American College of Rheumatology (ACR) guidelines on indications for first kidney biopsy

Increasing serum creatinine, without compelling alternative causes (such as sepsis, hypovolemia, or medication)
Confirmed proteinuria ≥ 1.0 g/24 h, or spot urine protein-to-creatinine ratio (uPCR) ≥ 1.0 mg/mg
Combination of: [i] proteinuria ≥ 0.5 g/24 h (or uPCR ≥ 0.5 mg/mg) plus [ii] hematuria ≥ 5 RBC per hpf, confirmed in ≥ 2 tests done within a short period of time, without compelling alternative causes
Combination of: [i] proteinuria ≥ 0.5 g/24 h (or uPCR ≥ 0.5 mg/mg) plus [ii] cellular casts, confirmed in ≥ 2 tests done within a short period of time, without compelling alternative cause (such as UTI, or menstruation)

Table 4 Revised 1995 World Health Organization (WHO) and 2018 Revised International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification of lupus nephritis [12, 33]

CLASS[b]	WHO 1995	ISN/RPS 2018
I	**Minimal change GN**[a]	**Minimal mesangial lupus nephritis** (mesangial immune deposits by IF or EM; normal by light microscopy)
II	**Mesangial GN**	**Mesangial lupus nephritis** (mesangial hypercellularity; no influx of leukocytes)
III	**Focal segmental GN** (proliferative, sclerotic lesions involving <50% of glomeruli)	**Focal lupus nephritis** (mesangial and capillary influx of leukocytes, or endocapillary wall immune deposits, involving <50% of glomeruli)
IV	**Diffuse proliferative GN** (DPGN, lesions involve >50% of glomeruli)	**Diffuse lupus nephritis** (mesangial and capillary influx of leukocytes, or endocapillary wall immune deposits, involving >50% of glomeruli)
V	**Membranous GN**, isolated (Va) or mixed with mesangial lesions (Vb)	**Membranous lupus nephritis** (sub-epithelial immune deposits without influx of leukocytes)
VI		**Advanced sclerosing lupus nephritis** (>90% glomerular sclerosis without active lesions)

[a] abbreviations: EM electron microscopy; GN glomerulonephritis; IF immunofluorescence
[b] Non-classifiable forms of lupus include lupus podocytopathy, pauci-immune renal vasculitis, renal thrombotic microangiopathy (TMA), and renal antiphospholipid syndrome (aPLS)

Table 5 Revised 2018 International Society of Nephrology/Renal Pathology Society (ISN/RPS) scoring system for disease activity and damage on kidney biopsy [12]

Activity index	Score[a]	Chronicity index	Score[a]
Endocapillary hypercellularity	0–3	Glomerulosclerosis	0–3
Glomerular neutrophils/karyorrhexis	0–3	Fibrous crescents	0–3
Fibrinoid necrosis	(0–3) × 2	Tubular atrophy	0–3
Hyaline deposits	0–3	Interstitial fibrosis	0–3
Cellular, fibrocellular crescents	(0–3) × 2		
Interstitial inflammation	0–3		
Total	0–24	Total	0–12

[a] Scoring of each criteria based on a 4-point scale: absent, mild, moderate, severe based on involvement of 0%, 1–25%, 25–50%, and >50%, respectively

Non-classifiable Kidney Manifestations

Some patients with SLE develop kidney disease that is not primarily caused by immune complex deposition. Lupus podocytopathy has been reported in 1% patients with adult onset SLE with kidney involvement [67]. These patients present with nephrotic syndrome; histology shows minimal change or focal segmental glomerulosclerosis (FSGS) with >50% podocyte foot process effacement on electron microscopy. The response to corticosteroids was inconsistent in patients with FSGS, but isolated lupus podocytopathy with minimal change histology tends to respond well to therapy with corticosteroids, with overall response rates of 95% at

12 weeks and complete response in 75%. As with podocytopathies in patients without SLE, relapse rates are high.

Pauci-immune lupus nephritis, with segmental necrotizing GN, has been described in SLE with class III-like histology, except that there was a paucity of immunostaining for immunoglobulin or complement, and paucity of electron dense deposits [138]. These patients are less likely to have hypocomplementemia or dsDNA antibodies, and more likely to present with AKI. Anti-neutrophil cytoplasmic antibodies (ANCA) are not specific for pauci-immune LN, but may be pathogenic in these patients. The prevalence of this overlap histology is low, at ~2% of all patients with lupus nephritis. Remission is reported in 50% patients, but renal and patient survival is worse than for class IV diffuse proliferative GN [69].

In inflammatory lupus nephritis, coincident microangiopathy often responds well to immunosuppressive medications. For isolated renal aPLS (▶ Chap. 21, "Autoantibodies and Kidney Diseases"), anticoagulation can be considered, especially when presenting with AKI. The diagnosis and management of isolated renal thrombotic microangiopathy (TMA) (Renal Involvement in Children with Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP)) is similar to atypical hemolytic uremic syndrome in patients without SLE.

Indications for Repeat Biopsy

Nephritic flares often warrant repeat biopsy to evaluate for proliferative lupus nephritis, especially when accompanied by high serum creatinine. Proteinuric flares are usually managed with an increase in the dose of either corticosteroids or maintenance immunosuppression, and a repeat biopsy is rarely necessary. Class change in lupus nephritis is commonly seen. The scenario to avoid is the patient with non-proliferative class or low activity index on initial biopsy who develops a kidney flare with undetected proliferative class or high activity [166]. Under-treatment of such patients can lead to non-responsiveness and kidney failure.

Laboratory Investigations

Multisystem involvement in cSLE necessitates a number of laboratory tests and ancillary procedures. The investigations are done primarily for diagnosis, as well as to monitor disease activity, response to therapy, adverse effects from medications, and guide to prognosis.

Urinalysis

Urinalysis is mandatory at baseline and periodically to examine for proteinuria, hematuria, and cylindruria. Urine protein-creatinine ratios (spot, 24-hour collection) are required for monitoring response to therapy [167].

Immunologic Studies

The most important are the serologic tests that detect antibody and complement levels. SLE is characterized by positivity of autoantibodies, the hallmark of which is ANA. ANA is used as a screening test; at a titer of 1:80 by immunofluorescence assay, it has an estimated sensitivity and specificity of 97.8% and 74.7%, respectively [85]. Although up to 33% of healthy children may have test positive to ANA, it is the best discriminator between children with SLE and those without chronic inflammatory disease. ANA pattern can be homogenous, diffuse, peripheral, speckled, nucleolar, or centromere. While all patterns are associated with SLE in different degrees, each pattern may also be associated with other autoimmune diseases. A homogeneous pattern is consistent with antibodies to native DNA and is commonly associated with SLE. The peripheral pattern, strongly associated with SLE, can be positive in autoimmune liver disease and Sjogren syndrome. Mixed connective tissue disease, scleroderma, overlap syndrome, and dermatomyositis exhibit a speckled, nucleolar, or centromere pattern.

Confirming the diagnosis of SLE in both children and adults requires further antibody testing. Anti-dsDNA antibodies are specific to SLE, associated with a homogeneous or peripheral ANA pattern, and predict active nephritis. Another specific, although poorly sensitive, lupus autoantibody is anti-Smith (Sm). It is associated with a speckled ANA pattern. Anti-ribonucleoprotein (RNP) is a nonspecific antibody, which when present in high titers is associated with mixed connective tissue disease. Anti-Ro/SSA and anti-La/SSB are characteristic of Sjogren syndrome and neonatal lupus [156].

Antiphospholipid antibodies (aPLs) are detected in about 30% patients with cSLE, significantly lower than in adults. The most important aPLs are anti-cardiolipin, lupus anticoagulant, and β2-glycoprotein-I antibodies. Presence of lupus anticoagulant causes prolonged aPTT. Anti-cardiolipin and β2-glycoprotein-I antibodies have three subcomponents, IgG, IgA, and IgM, with IgG being the most important clinically. Antiphospholipid syndrome is characterized by recurrent arterial or venous thrombotic events and complications during pregnancy. Trauma, surgical procedures, and infections may transiently cause positive aPLs.

Complement levels have been used as markers of disease activity; hypocomplementemia often signify active lupus especially lupus nephritis. The complement system appears to have protective features in that hereditary homozygous deficiencies of the classical pathway components, such as C1q and C4, are associated with increased risk for SLE [37]. Hence, it may be difficult to know whether the low complement is due to consumption or from inherited deficiencies of one or more alleles.

Extrarenal Manifestations

Laboratory evaluation of cSLE should include determination of acute phase reactants, complete blood count, and biochemical profile. Erythrocyte sedimentation rate and C-reactive protein are indirect parameters of inflammation and can be useful in differentiating lupus flare from infection. Complete blood count is advised to screen for potential cytopenias; anemia must be evaluated with peripheral smear, reticulocyte count, iron studies, and Coombs test. A chemistry panel will evaluate electrolytes, transaminases, and kidney function. Patients should be evaluated for risk factors for atherosclerosis with fasting blood glucose and lipid profile. Thyroid function tests, liver function tests, and bilirubin levels are determined, and appropriate biopsies performed [91].

Chest radiographs will detect cardiomegaly, active pulmonary infiltrates, and serositis. Work up for cardiopulmonary complications entails doing pulmonary function testing with lung diffusion testing, two-dimensional echocardiography, and high-resolution computed tomography (CT) scans [124]. Magnetic resonance (MR) imaging of the brain may be useful for evaluation of neuropsychiatric lupus, or of joints in cases of avascular necrosis. MR or CT angiography will assess for thrombi for lupus-related thrombosis.

Cognitive function testing particularly for memory and intelligence should be considered in cSLE, either if behavioral changes occur, or school performance declines. The Weschler Abbreviated Scale of Intelligence and Delis-Kaplan Executive Function System can be used for this purpose [79]. Dual-energy x-ray absorptiometry is used to evaluate bone mineral density in the background of long-term glucocorticoid use [151].

Prognostic Factors

Predictive laboratory factors for long-term kidney outcomes at early stages have been studied. These investigations are pertinent to define clinically relevant short-term and long-term targets of therapeutic intervention [116]. Early decrease in 24-h proteinuria (<0.8 g/day) is the best predictor of long-term kidney outcome in patients with SLE. Another study reported that decreased proteinuria at 12-months follow-up was the best predictor of kidney outcome at 7 years for an ethnically distinct group of patients with severe lupus nephritis [159]. Notably, these studies also showed that the addition of other variables, such as level of serum creatinine and hematuria, did not improve the predictive value of 24-hours proteinuria [159].

Models have been developed that predict long-term outcomes in lupus nephritis using patient response data following therapy. Both proteinuria and serum creatinine levels at 1 year were important components for these models; urinalysis was not shown to add value [96]. In cSLE, low level of complement C3 in conjunction with high serum creatinine is associated with progressive kidney failure. A recent review on disease severity and progression, including data from 26 studies showed that high levels of creatinine (>1.5 mg/dL) at disease onset was a predictor for progression to kidney failure [97].

Prospective analysis of prognostic factors shows that anti-dsDNA antibodies did not predict a flare. High titers of antibodies specific for dsDNA using *Crithidia* immunofluorescence can predict with modest accuracy the presence of lupus nephritis (sensitivity 57%, specificity 97%) [77].

There are promising combinations of urinary and serum non-invasive biomarkers to predict long-term outcome and to estimate the degree of irreversible and chronic kidney damage in patients with lupus nephritis [167]. Urinary levels of both neutrophil gelatinase-associated lipocalin (NGAL) and monocyte chemo-attractant protein-1 (MCP-1) are predictive of renal outcomes [163]. Another prospective study, which evaluated ten urinary biomarkers in pediatric patients with lupus nephritis, showed that decline of GFR was associated with persistent high levels of TGFβ, transferrin, and LFABP, indicating continued renal inflammation [25].

Management

Trials in adults have informed guidelines for treatment of lupus nephritis in children and adolescents [43, 51, 78, 103]. Treatment is geared to promote remission, limit disease exacerbations/flares, and prevent kidney failure. Response definitions vary between studies, but cSLE definitions have been proposed (Table 6). Management of lupus nephritis is based on disease severity and risk of progressive kidney damage.

Lupus nephritis class I and II are associated with good prognosis. Conservative treatment (see below) is recommended. Although no additional immunosuppressive therapy is needed for the nephritis, patients require continued monitoring. The development of worsening proteinuria or elevated creatinine may indicate conversion to other nephritis classes. Class III, IV, V, and mixed (III/IV + V) class lupus nephritis requires therapy with systemic immunosuppression in addition to high-dose corticosteroids. Aggressive therapy is necessary when children present with nephrotic syndrome, or for findings on kidney biopsy of active cellular proliferation and necrosis involving ≥40% of glomeruli. Two treatment phases are utilized: (i) Induction, which lasts on average 3 to 6 months; and (ii) Maintenance where immunosuppressive treatment is reduced over time [56, 129]. Lupus nephritis class IV is the most severe form of nephritis and requires aggressive therapy. The long-term effect of

Table 6 Definitions of kidney outcomes in patients with cSLE and lupus nephritis [103]

Outcome measure	Definition
Core renal parameters	Proteinuria (spot protein to creatinine ratio)
	Kidney function (creatinine clearance, serum creatinine)
	Urine sediment (leukocytes, red cells, or casts)
Renal response definitions	
Substantial response (complete remission)	Inactive urine sediment (<5 leukocytes and < 5 red cells/hpf; no casts) Urine protein to creatinine ratio < 0.2 Normal kidney function (estimated GFR >90 mL/min/1.73 m^2)
Moderate response	>50% improvement in 2 of 3 core renal parameters (maximum protein to creatinine ≤ 1.0), without clinically relevant worsening of the remaining parameter
Mild response	30–50% improvement in 2 of 3 core renal parameters, without clinically relevant worsening of the remaining core parameter
No response	Patients who do not qualify for any improvement as above

GFR glomerular filtration rate, *hpf* high powered field remission after treatment depends upon response to therapy [81]. In 86 patients followed for 10 years, compared to nonresponders, those achieving complete remission had superior patient survival (95% vs. 60%) and renal survival (94% vs. 31%).

Initial Therapy for Proliferative Lupus Nephritis

Corticosteroids form the foundation of therapy. While first-line for rapid control of inflammation, time- and dose-dependent treatment-related toxicity exists. Maximum doses of oral corticosteroids of 2 mg/kg/day (60 mg/day) are advised. Pulse methylprednisolone is effective in treating severe, active disease and allows for rapid reduction of oral prednisone doses. An observational study examined the efficacy and safety of repeat pulse methylprednisolone on response rates and in prednisone sparing in lupus nephritis [134]. The optimal starting dose and oral regimen are not well established. For guidance on initial therapy, consensus plans have been developed. Corticosteroid strategies include primarily oral or primarily IV routes, and mixed IV and oral route [103].

The original 1980 NIH study laid the foundation for cyclophosphamide (oral or IV) use as first-line therapy for induction. Patients randomized to prednisone and intermittent IV cyclophosphamide (0.5 to 1 g/m^2 monthly for 6 doses) had superior renal survival, compared to azathioprine (AZA) plus prednisone, or prednisone alone. Lower cumulative exposures with IV compared with oral cyclophosphamide resulted in fewer cytopenias, less hemorrhagic cystitis, and better adherence [9]. Subsequent efforts to lower cyclophosphamide exposure and reduce side effects led to the Euro-Lupus nephritis trial. Ninety patients with proliferative lupus nephritis were randomized to receive either the NIH cyclophosphamide regimen or a low-dose IV regimen (500 mg every 2 weeks for 6-doses). Not only was no difference seen in kidney outcomes at 41 months or at 10 years, but also less toxicity and fewer severe infections occurred with the lower dose regimen [66]. Efficacy of IV cyclophosphamide (NIH protocol) was also seen in Polish [153] and US [84] children with lupus nephritis class III and IV followed for 32 and 36 months, respectively. Kidney biopsies completed before and after treatment showed decreased activity index without increased chronicity index. Significant improvement was observed in creatinine clearance, urine protein excretion, hypertension, and SLEDAI score, allowing for >50% reduction in corticosteroid dose.

Although the ACCESS trial of abatacept, a CTLA4-Ig construct/fusion protein that blocks the CD28/CD80 costimulatory pathway for T-cell activation, did not show additive benefit for initial treatment of lupus nephritis [1], the use of low-dose Euro-lupus protocol for cyclophosphamide has resulted in a major change in initial treatment guidelines [129, 168]. At 12 months, patients in the placebo arm who received IV cyclophosphamide (500 mg every 2 weeks) achieved a complete renal response rate of 46% (overall response rate 64%) [1]. Approximately 40% patients in the ACCESS trial were black and 40% were Hispanic: these patient populations had not been widely represented in the European trials [66]. However, children under 18 years were not represented.

Mycophenolate mofetil (MMF) is an effective initial agent for lupus nephritis in adults and children [41]. Multiple trials have demonstrated comparable efficacy of MMF to cyclophosphamide, including the landmark trial of 140 multiethnic patients treated with MMF (1 g to 3 g/day) or monthly IV cyclophosphamide (0.5 to 1 g/m^2). Not only was remission significantly more frequent in the MMF group after 6 months, but the safety profile was also more favorable. The Aspreva Lupus Management Study (ALMS) was a multiethnic international prospective study of 370 patients with proliferative lupus nephritis, comparing MMF (3 g/day) to cyclophosphamide for induction [5]. At 6 months, the response rate (complete and partial response) was 56% with the former and 53% with the latter. By 3.5 years, complete responses were seen in 62% of MMF and 59% of cyclophosphamide treated subjects. Both treatments had similar adverse events, with more gastrointestinal side effects with MMF.

Subgroup analysis, in black and Hispanic patients, demonstrated better response rates with MMF (60%) compared to cyclophosphamide (39%) [5]. While a meta-analysis showed similar renal response for cyclophosphamide and MMF in class IV nephritis [71], data on effectiveness in the severe forms of the illness is limited.

In cSLE with proliferative LN, a 51 patient UK cohort treated with corticosteroids and either MMF or cyclophosphamide showed equivalent reductions in proteinuria, serum creatinine and disease activity scores at 8 and 14-months follow-up [145]. Time to kidney flare was 343–451 days in cyclophosphamide and MMF groups. Parameters of disease activity (ESR, anti-dsDNA antibodies, C3) also improved in both groups. The recommended induction dose of MMF in children is 1200 mg/m^2/day, and may be adjusted according to tolerance, efficacy, and trough MPA blood levels.

Monoclonal antibodies targeting CD20 expressed on B cells have demonstrated clinical improvement of lupus nephritis in many observational studies. Significant improvements in disease activity, anemia, autoantibody titers, hypoalbuminemia, and proteinuria were reported in 63 children treated with 104 courses of rituximab during a 10-year period [164]. The Lupus Nephritis Assessment with Rituximab (LUNAR) study was a large, global, phase III, randomized, placebo-controlled trial comparing rituximab added to MMF and corticosteroids for induction therapy of proliferative lupus nephritis [128]. After 1 year, while there was no significant difference in complete or partial response (57% vs. 46% with placebo, $P = 0.18$); post-hoc analysis associated extent of peripheral B-cell depletion with complete remission. Among patients who achieved complete B-cell depletion, 47% achieved complete remission, compared with 13% of study participants with incomplete depletion [50]. The duration of B-cell depletion was also associated with better response. Nonsignificant trends were also reported in complete renal response rates at 3 and 12months in an Italian cohort of 54 patients with proliferative lupus nephritis (71% rituximab, 65% cyclophosphamide, 53% MMF) [109]. Studies on newer anti-CD20 agents, for example, ofatumumab and obinituzumab, also appear to be beneficial in patients who achieve complete B-cell depletion.

Belimumab is a monoclonal antibody that suppresses and inhibits the activity of B-cell activating factor (BAFF), also known as BLyS (B-lymphocyte stimulator). The soluble form of BAFF is overexpressed in active lupus nephritis. Belimumab was superior to placebo in 1684 non-renal adult onset patients with SLE, as add-on therapy (to MMF, cyclophosphamide, or azathioprine) [110]. A post-hoc analysis on 52-week outcomes revealed improved kidney outcomes: kidney flare rates, proteinuria, and kidney function. In the global lupus nephritis-BLISS phase III trial of 448 patients, belimumab improved 2-year response rates, with a number needed to treat of 9 [45]. Modification of induction protocols based on belimumab trials are likely to occur, as pediatric data is published.

Calcineurin inhibitors (cyclosporine, tacrolimus, voclosporin) have immunomodulatory and nonimmune-mediated roles in treatment. They inhibit T-cell proliferation as well as reduce proteinuria, through stabilization of podocyte cytoskeleton and afferent arteriole vasoconstriction [17, 104]. A randomized trial on 60 Asian patients with lupus nephritis class III, IV, and V reported similar 6-month complete response rates to therapy with steroids and either tacrolimus (75%), MMF (70%), or IV cyclophosphamide (60%) [89]. Serum albumin increased and proteinuria decreased faster in the tacrolimus cohort. A larger randomized controlled trial of 150 patients assigned to initial therapy with either tacrolimus or MMF reported 6-month complete response in 62% and 59%, respectively [106]. However, at median follow-up of 61 months, there was non-significant trend toward more nephritis flares with tacrolimus (54%) compared to MMF (38%). The incidence of a cumulative composite outcome (decline in creatinine clearance ≥30%; advanced CKD; death) was similar for both groups [106].

Calcineurin inhibitors have been studied in a multi-targeted approach to initial therapy for proliferative lupus nephritis. In a randomized controlled trial of 368 patients also treated with IV methylprednisolone and prednisone, the overall response was higher in patients receiving 24 weeks of MMF (1 g/day) plus tacrolimus (4 mg/day) than those receiving IV cyclophosphamide by the NIH protocol (84% vs. 63%) [92]. Voclosporin, an analog that does not require drug-level monitoring, has been studied in a phase 2 trial of induction therapy, in combination with MMF (2 g/day) and rapid steroid tapering. Among 265 geographically diverse patients at 6 months, the multi-target therapy achieved superior remission rates (27–33%) over placebo (19%) [130]. Higher complete renal remission rates persisted at 12 months, although increased rates of adverse events occurred with voclosporin. Initial results from the phase 3 AURORA trial of voclosporin are encouraging. A meta-analysis of 45 induction trials involving 4222 patients with proliferative disease, including children, showed higher remission rates with MMF, calcineurin inhibitors, and multi-target therapy than with IV cyclophosphamide [115]. Although longer-term outcomes are needed, calcineurin inhibitors are a satisfactory option for some patients with lupus nephritis.

Although no longer considered first line, azathioprine has been shown to be effective in select patients. In a Dutch study, 87 patients with proliferative LN responded similarly to oral steroids and either IV cyclophosphamide (750 mg/m^2; 13 pulses in 2 years) or azathioprine (2 mg/kg/day) combined with IV methylprednisolone [53]. However, after 5 years, relapses were higher in the latter group.

Initial Therapy for Membranous Nephropathy

Due to the favorable prognosis of adults with membranous LN with sub-nephrotic proteinuria, no additional immunosuppressive therapy is indicated and anti-proteinuric measures (section "Prognostic Factors") alone are recommended. However, pure membranous LN with nephrotic-range proteinuria is associated with thrombosis, doubling of serum creatinine in 20% and kidney failure in 25% of affected children [119]. In cSLE, consensus guidelines recommend corticosteroid and nonsteroidal immunosuppression in all patients.

In lupus nephritis class V, the first-line agent for immunosuppression is unclear. In 42 patients enrolled in a randomized trial of nephrotic-range proteinuria (age 13–60 years), remission rates at 1 year were superior with cyclosporine (83%), followed by cyclophosphamide (60%) and prednisone alone (27%) [10]. However, on long-term follow-up of 103 patients, kidney outcomes were nearly identical (95% complete response rate and 10-year renal survival of 86%) using corticosteroids with either oral cyclophosphamide, chlorambucil, azathioprine, cyclosporine, MMF, or methotrexate [108]. The Kidney Disease Improving Global Outcomes guideline recommends therapy with either MMF, cyclophosphamide, cyclosporine, or rituximab [78].

In an open-label trial of azathioprine and prednisone, complete remission occurred in 67% of 38 patients at 12 months, with an overall response (complete and partial remission) of 89% [105]. The cumulative 10-year incidence of kidney flares was 32%, and 16% had $\geq$30% decline in GFR. Better long-term renal survival was seen with steroids and cyclophosphamide, compared to steroids alone [9]. A randomized trial on 19 patients with 83 ± 59 months follow-up reported 91% response with corticosteroids plus chlorambucil alternating over 6 months, compared to 50% with corticosteroids alone [107].

A pooled analysis on two large randomized trials of MMF ($n = 33$) and IV cyclophosphamide ($n = 32$) reported no difference in proteinuria and serum creatinine at 24-weeks follow-up. Subgroup analysis of patients with nephrotic syndrome showed no difference in response rates [123]. Another study found that 6 of 10 patients treated with cyclosporine had reduction in proteinuria <1 g/day and two had reduction to 1–2 g/day at 43-months follow-up [122]. While half the patients had decrease in activity index on repeat biopsy, a rise in chronicity index was common. In another randomized trial on 28 patients with pure membranous nephropathy, better response at 6 months was reported with corticosteroids and tacrolimus, than with steroids and MMF (100% vs. 75%; $P = 0.09$) [106]. Relapses were common after cyclosporine was stopped. In 40 patients, multi-target induction therapy using corticosteroids plus MMF with tacrolimus achieved complete remission at 6 months in 50%, compared to 5% with IV cyclophosphamide [14]. There was no increase in adverse events in patients receiving multi-target therapy.

Maintenance Therapy

Following completion of induction therapy, maintenance therapy is required. Without it, flares are common and portend worsening kidney function, kidney failure, and even death [118]. Thus, the goals of maintenance therapy are to sustain remission while avoiding the toxicity of treatment regimens, and to prevent relapses and flares. Research supports use of MMF and azathioprine, but the duration of maintenance therapy is not evidence-based. Expert opinion suggests continuation for 2 to 3 years after complete remission of lupus nephritis is established. Repeat kidney biopsy showing histologic remission during maintenance may help in the decision-making to taper off therapy.

MMF has emerged as the therapy of choice for maintenance, although AZA is an alternative particularly in pregnant patients. A prospective comparison of three drugs following cyclophosphamide-induced remission of class III or IV lupus nephritis was performed in 54 patients [34]. Patients were randomized to quarterly pulse cyclophosphamide (0.5–1 g/m^2), azathioprine (1–3 mg/kg/day), or MMF (500–3000 mg/day). Event-free survival for the composite endpoints (death and kidney failure) at 72 months was significantly higher with MMF and AZA than IV cyclophosphamide. Five deaths (four in cyclophosphamide group; one in MMF group) and five kidney failure events (three with cyclophosphamide; one each with MMF and azathioprine) developed during follow-up. Relapse rate was significantly lower in MMF compared to the cyclophosphamide group, as were the adverse events [34].

In the European MAINTAIN proliferative lupus nephritis trial, 105 patients managed by Euro-lupus cyclophosphamide induction were assigned to MMF (2 g/day) or azathioprine (2 mg/kg/day) [65]. At 48-months follow-up, nephritis flares were similar (19–25%) with MMF or azathioprine, as were activity and chronicity indices on repeat biopsy. The multiethnic ALMS maintenance trial compared azathioprine (2 mg/kg) and MMF (2 g/day) in 227 patients, following induction therapy with either MMF or IV cyclophosphamide. Composite outcomes including death, kidney failure, doubling of serum creatinine, flares, or need for rescue therapy were two-fold lower with MMF (16% vs. 32%) [42]. Serious adverse events were similar between the two groups.

Calcineurin inhibitors have been proposed as maintenance therapy. Compared to azathioprine, cyclosporin was non-inferior based on rate of flares, preservation of kidney function, and reduction in proteinuria. In 70 patients who achieved remission with induction therapy, tacrolimus and azathioprine achieved similar rates of relapse [32]. A study on 16 patients showed comparable reduction of proteinuria, and similar blood levels of albumin and creatinine following therapy with tacrolimus and MMF at 106 weeks [171]. Similarly, a Japanese study on 26 patients treated with tacrolimus (3 mg/day) maintenance therapy and followed for 5 years demonstrated reduced lupus activity and stable GFR [76].

The optimal dose and duration of corticosteroid therapy during the maintenance phase is not clear. In a placebo-controlled randomized trial that examined the feasibility of such withdrawal, prednisolone was discontinued in seven patients and continued at low dose in eight. Over the next 36 months, renal flare occurred in one patient in former group, compared to four in the latter (three renal flare; one major extrarenal flare) [46].

Conservative Management

Non-immunosuppressive therapy is imperative to reduce the risk of kidney failure. Renin-angiotensin-aldosterone blockade is used for its anti-proteinuric and antihypertensive effects, including in adult-onset disease [73]. The blood pressure should be controlled below the 90th percentile for age, sex, and height. Lipid levels should be monitored. Routine use of statins had no clinically significant reduction in carotid intima-media thickening [139]. Nephrotoxic drugs should be avoided. Bone protection should follow non-pharmacologic and pharmacologic measures, particularly with prolonged corticosteroid use.

Adjuvant therapy with hydroxychloroquine (HCQ) or chloroquine reduces the risk of flares of lupus nephritis, kidney failure, and death. A systematic review showed strong evidence supporting their use in preventing flares and promoting patient survival [133]. In a longitudinal cohort of 459 patients administered HCQ or chloroquine for ~5 year, patients showed significantly reduced disease activity, decreased orbidity, and reduced cumulative corticosteroid dose [114]. Additional benefits include improved lipid and glucose metabolism, reduced risk of thromboembolism and cardiovascular events, and fewer infections and malignancies [133]. Although HCQ is well tolerated, there is small risk of maculopathy and loss of visual acuity. Risk factors for maculopathy include HCQ dose >5 mg/kg/day, duration of use >5 years, impaired kidney or liver function, and underlying macular disease. The mean maintenance dose of HCQ in adults is 200 or 400 mg/day. Appropriate ophthalmologic screening is advised at start of therapy, and then annually or biannually depending on risk factors.

Refractory Lupus Nephritis

KDIGO defines complete and partial remission to assess response to treatment. Complete remission is return of kidney function to baseline, and decline in urine protein/creatinine to <5 mg/mg. Partial remission requires stable or improved kidney function, and ≥ 50% reduction in urine protein/creatinine ratio [78]. Treatment-resistant or refractory disease is identified by failure to improve within 3–4 months, absence of partial remission after 6–12 months, or failure of complete remission after 2 years. Within that framework, patients who fail to respond to initial treatment with MMF or cyclophosphamide may be switched to the alternate therapy. Of 85 patients with refractory or relapsing nephritis after cyclophosphamide as initial therapy, 87% responded to MMF within a median time of 3 months [126]. In 17 MMF-resistant patients, adding tacrolimus resulted in significant reduction in proteinuria and 70% response rates at 2 years [36]. In 21 patients with refractory disease, multitargeted therapy using MMF, cyclosporine, and corticosteroids achieved response in 70% patients within 1 year [75]. Open label investigations also support a role for rituximab [165]. Of 22 patients with refractory class III or IV nephritis, add-on therapy with rituximab (0.5 to 1 g on days 1 and 15) was associated with satisfactory response in 12 [162]. In a meta-analysis of 223 patients treated with rituximab for refractory illness, 51% had complete and 27% had partial remission [3].

Intravenous immunoglobulins (IVIg) have been studied in lupus nephritis and for nonrenal features of SLE. Evidence from few trials and observational studies suggests that IVIg lowers levels of dsDNA antibodies, with reduction in proteinuria and improvement in kidney function [113]. While therapy with IVIg and subcutaneous immunoglobulin [21] are effective in some patients with refractory lupus nephritis, most trials were uncontrolled. Due to limited availability of IVIg, its use tends to be reserved for patients with hypogammaglobulinemia, resulting either from active lupus or from the effect of medications.

Plasmapheresis has been reported to be successful in anecdotal cases, and is believed to remove circulating immune complexes. Plasmapheresis added to oral cyclophosphamide and prednisone was compared to therapy without plasmapheresis in severe lupus nephritis [88]. At mean follow-up of 136 weeks, there was no difference between the groups in terms of renal and patient survival. Although not justified for routine use, plasmapheresis may be helpful in patients with refractory antiphospholipid antibodies and contraindications to anticoagulation, or those with both positive lupus and ANCA serology.

Relapses of Lupus Nephritis

Of patients who achieve complete or partial remission, kidney relapses occur in 27–66%

[49]. Relapse of nephritis is a predictor for development of kidney failure. Relapses arise due to medication non-adherence, but may occur with altered maintenance immunosuppression. The diagnosis is based on clinical criteria and includes worsening lupus serology (decrease in C3 and C4, increase in anti-dsDNA antibodies), increasing proteinuria, active urine sediment, and increase in serum creatinine from baseline [103]. Active lupus serology and/or urine sediment requires close monitoring as they are associated with relapse of nephritis. Proteinuric relapses are managed with increased maintenance immunosuppression or steroid dose. Repeat kidney biopsy aids in identification of class switch from non-proliferative to proliferative LN in the presence of worsening urinary findings. This histologic conversion can be found in 40–76% of cases following relapse [51]. Treatment is tailored based on kidney biopsy findings. Active lesions with a nephritic relapse require a change in immunosuppression, using an agent with different mechanism of action, intensified therapy, and/or increased dose of steroids. A less aggressive approach is indicated in patients with pure membranous LN.

Extrarenal Disease

The management of SLE is challenging due to chronic relapsing and remitting course, wide spectrum of features from mild to life-threatening conditions, and multisystem involvement with varying severity. The goals of treatment are thus to: (i) maintain low degree of lupus activity using immunosuppressive agents, and avoid known triggers, (ii) prevent organ damage, (iii) reduce comorbidities secondary to lupus and its treatment, and (iv) address fatigue and pain, which often are not associated with active lupus.

Glucocorticoids remain the standard therapy for nonrenal SLE, the dose and duration being dependent on the severity of manifestations. HCQ is recommended due to beneficial effects on arthritis and cutaneous lesions, reduced risk of disease flares, and better cardiac outcomes [133]. Immunosuppressive agents are indicated for persistent disease activity, and as steroid-sparing agents to maximize control of disease activity while minimizing drug-related-morbidity. There is lack of randomized controlled trials that compare the efficacy and safety of different immunosuppressive agents in cSLE. For mild to moderate SLE, methotrexate, azathioprine, and MMF are commonly used, while cyclophosphamide and rituximab are indicated for life-threatening disease [43]. IVIg and rituximab have shown benefit in therapy of severe thrombocytopenia with inadequate response to high dose glucocorticoids [95].

Geographic, Race, and Ethnic Considerations

Different ancestries and ethnicities display different disease characteristics, disease activity/damage scores, and outcomes in cSLE populations around the world [48, 61, 86, 87]. Poverty, low educational status, lack of health insurance, and poor social support, which are more prevalent in non-white populations, have negative impact on disease course and outcomes [29]. Therefore, cSLE management should consider the impact of racial/ethnic background, as well as costs and availability for immunosuppressive and biological agent use.

Two cohorts of Amerindian ancestral cSLE and adults SLE patients, GLADEL (Latin American Group for the Study of Lupus) and LUMINA (Lupus in Minorities: Nature vs Nurture), showed poor outcomes compared to patients of Caucasian ancestry, possibly related to genetic background and socioeconomic factors [158]. Recently, the first Latin American clinical practice guidelines for the treatment of SLE: GLADEL and Pan-American League of Associations of Rheumatology (PANLAR) was published. Pharmacologic options were examined for pediatric and adult lupus nephritis, as well as musculoskeletal, mucocutaneous, cardiac, pulmonary, neuropsychiatric, and hematological involvements and the antiphospholipid syndrome. While more African American and Hispanic patients responded to MMF than cyclophosphamide in adult SLE, there is reduced availability of MMF and tacrolimus in many Latin American countries, making cyclophosphamide the best option for induction. For pediatric lupus nephritis, the panel of pediatric rheumatologists also suggested IV cyclophosphamide for induction, despite the risk of ovarian and testicular toxicities, and azathioprine for maintenance treatment.

Geographical considerations for lupus nephritis have been also reported by Asian populations. Relatively low frequency of disease flares was observed in Chinese patients receiving maintenance with low-dose prednisolone and MMF [170]. Calcineurin inhibitors are also treatment options for pediatric LN. In real-world practice, initial data on tacrolimus treatment came chiefly from Asian children. Therapy with tacrolimus seems promising for maintaining and re-inducing remission in Japanese cSLE patients [155].

Outcomes

Activity and Damage

The challenge of disease activity and need for estimating damage has led to development of different disease tools [142]. These instruments are reliable and valid for use in cSLE, each with similar psychometric properties. The overall disease activity scores assessed in practice are the British Isles Lupus Assessment Group (BILAG), the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), and the Physician's Global Assessment of Disease Activity (MD Global). There is consensus that all patients be regularly evaluated for disease activity, using either SLEDAI 2000 (SLEDAI-2K) or pediatric BILAG 2004 [52]. Due to simplicity, feasibility, and good metrologic properties, MD Global should also be included for disease activity in clinical practice and research [54].

Disease activity should be measured at disease diagnosis and during disease course. Data from the Childhood Arthritis and Rheumatology Research Alliance (CARRA) showed delays in diagnosis of 3 to 12 months in 23% and severe delay of >1 year in 9% of 598 cSLE patients. No significant differences were observed in SLEDAI among children and adolescents with rapid compared with moderate or severe delay of cSLE diagnosis [131]. The Brazilian cSLE Registry evaluated three different groups with distinct time intervals between onset of signs/symptoms and disease diagnosis in 1555 cSLE patients: short time interval to diagnosis (<1 month, 4%), intermediate time interval (≥1 and < 3 months, 33%), and long time interval (≥3 months, 63%). The median SLEDAI-2 K was significantly higher in patients with short time interval to diagnosis compared to the other groups, indicating more active and multisystem presentation [112].

The course of illness in cSLE is characterized by episodes of disease activity requiring intensive drug therapy, followed by periods of improvement. An international consensus defines cSLE flares as "measurable worsening of disease activity in at least one organ system, involving new or worse signs of disease that may be accompanied by new or worse SLE symptoms; depending on the severity of the flare, more intensive therapy may be required" [23]. Consensus criteria for global flares in cSLE define thresholds for minor, moderate, and major flares. These criteria include the most relevant disease activity parameters, and are important for gauging systemic responses to interventions intended to treat systemic or organ-specific manifestations of cSLE [26]. An intervention that is effective in reducing proteinuria in cSLE patients with lupus nephritis would not be desired if at the same time it evoked new onset mucocutaneous or neuropsychiatric lupus.

An equally important outcome measure is the SLICC/ACR damage index (SDI) score. The SDI describes cumulative damage and likely reflects disease activity and severity over time. High scores indicate more severe organ damage. The commonly scored items scored are proteinuria, scarring, alopecia, and cognitive impairment [64]. High-impact manifestations are weighted: myocardial infarction, stroke, avascular necrosis, malignancy, significant tissue loss, gastrointestinal infarction or resection, and dialysis dependence [142]. SDI score is predominantly disease-related, although side effects of high dose glucocorticoids and immunosuppressive drugs also contribute [142]. European recommendations suggest yearly evaluation using SDI score [52].

The SDI has been validated in cSLE [142]. Accrual damage may have long-lasting physical and psychosocial issues for cSLE patients. A pediatric version of SDI is available, with two pediatric-specific domains: growth failure and delay in development of secondary sexual characteristics (observed in 15% and 11% of patients, respectively) [55]. In cSLE, cohorts followed 2–4 years, the frequency of any damage (SDI >1) ranges from 28% to 52% [64]. Afro-Caribbean ancestry and major organ and system involvements (lupus nephritis class III/V, cerebrovascular accidents, vasculitis, alveolar hemorrhage, or myocarditis) were associated with greater damage. A study of 111 cSLE patients showed median time to stroke, kidney transplant, replacement arthroplasties, and myocardial infarction at 20, 24, 34, and 39 years, respectively [51].

Consequences of cSLE therapy or disease activity may contribute to specific damage in children and adolescents, especially infections, gonadal dysfunction/infertility, and osteoporosis [142]. Infections are an important cause of morbidity, and increase cumulative damage, as reported with invasive fungal infections (candidiasis, aspergillosis, cryptococcosis, histoplasmosis, and paracoccidioidomycosis). Risk factors of infection include disease activity and duration, lymphopenia, leukopenia, neutropenia, hypocomplementemia, functional asplenia, and therapy with immunosuppressive agents [142]. Primary immunodeficiency (C2, C4, C1q, C1r-s, IgG2, IgM, and IgA deficiencies) was identified in 22% of cSLE patients and contributes to risk of systemic infections. SDI scores were higher in patients with primary immunodeficiency, compared to those without these conditions [70].

The term infertility is generally inadequate for adolescents with cSLE, since infertility is defined as inability to have a child after 1 year of sexual activities without the use of contraception [142]. Therefore, the nomenclature gonadal (ovarian or testicular) dysfunction is appropriate for adolescent and youth SLE populations. Global evaluation of ovarian reserve comprises new markers of ovarian function: anti-Müllerian hormone levels and ovarian ultrasound to assess antral follicle count. Two studies assessed these parameters in adolescents and young female cSLE patients. Decreased ovarian reserve markers, in spite of presence of regular menstrual cycles, were observed in cSLE patients after intravenous cyclophosphamide use [2]. Another study evaluated ovarian reserve parameters and anti-corpus luteum antibodies in adult SLE patients with pediatric onset, and showed that a high cumulative methotrexate dose was a possible cause of subclinical ovarian dysfunction for this population [40].

Overall testicular function was systematically assessed in 30 adolescents and adults with SLE and 30 healthy subjects. Low sperm count, low motile sperm, low normal sperm forms, reduced testicular volumes, and elevated follicle stimulating hormone levels occurred significantly in SLE patients compared to healthy controls, with similar frequencies of anti-sperm antibodies. The chief contributing factor for these testicular abnormalities was the intravenous cyclophosphamide use. The duration of time from the last dose of this alkylating therapy was 5.1 ± 3.7 years, indicating persistent or long-lasting damage to primordial sperm cells, and sperm abnormalities [147].

cSLE patients may also have a significant impact on bone health and a permanent reduced bone accrual over years, resulting in a reduction of bone mass peak and a high risk of osteoporosis in early adulthood [142]. Low aerobic fitness, muscle weakness, chronic inflammation, glucocorticoid use, pubertal delay, and reduced intake of calcium and vitamin D may be responsible for reduced bone mineral density in cSLE patients [117].

SLE on Dialysis

The mortality rate on dialysis (22% at 5 years) is similar to that reported for other causes of pediatric-onset kidney failure [62]. Patients with SLE have similar outcomes on chronic dialysis with either hemodialysis or peritoneal dialysis [31]. Due to concerns for peritonitis, some centers prefer hemodialysis for patients on high dose immunosuppression [74]. Peritoneal dialysis may be preferable in patients with antiphospholipid antibody syndrome due to the possibility of hemodialysis access failure from thrombosis [141].

The 10-year survival rate for SLE patients with CKD5 is better for those taking prednisone and hydroxychloroquine than corticosteroids alone, and outcome of dialysis is worst for patients on no immunosuppressive medication [22]. Whereas azathioprine and cyclophosphamide are cleared with hemodialysis, the dialytic clearance (▶ Chap. 86, "Drug Dosing in Abnormal Kidney Function in Children") of other immunosuppressive drugs is minimal [98]. Cyclophosphamide dose is usually reduced 50% and given one day before the next hemodialysis session [8]. As opposed to patients in the ICU with oliguric AKI who receive IV cyclophosphamide, there is no need for intravenous fluids or bladder irrigation for anuric patients with kidney failure. There is no data on clearance of immunosuppressive agents during peritoneal dialysis.

Patients should continue to be followed by their rheumatologist after declaration of kidney failure, as extrarenal flares are common. SLE patients on dialysis who continued to have two or more follow-up visits annually with rheumatologists had improved longevity and were more likely to receive effective immunosuppressive therapy [22].

SLE Following Transplantation

One-third of cSLE patients with lupus nephritis and kidney failure receive a kidney transplant within 5 years. Based on data from the US Renal Data System (USRDS) from 1995 to 2006, 51% were African American and 24% Hispanic [62]. There were significantly fewer kidney transplants amongst adolescents (as opposed to younger children), black and Hispanic children, and those with Medicaid (compared to private insurance). Mortality from SLE with CKD5 is almost double among blacks compared to whites [62].

Serological markers of disease activity (complement C3 and C4; dsDNA antibodies) are even less accurate measures of disease activity during the post-transplantation period. While glomerular immune complex deposition is seen on half of surveillance biopsies, recurrent lupus nephritis is very low: less than 3% of patients had symptomatic disease [35]. Overall, graft survival and infection-related complications are comparable between transplant patients with lupus nephritis-associated kidney failure and with other causes (▶ Chap. 84, "Renal Transplantation: Non-infectious Complications and Long-Term Outcome") [35, 62]. Only 7% of graft failures are attributable to recurrent disease (Renal Transplantation: Disease Recurrence). However, patients with recurrent nephritis have a fourfold increased risk for graft failure [35].

A history of APLS is associated with reduced 10-year graft survival. To a lesser degree, the presence of antiphospholipid antibodies in SLE patients is also a risk for early graft failure. Anticoagulation with warfarin or low molecular weight heparin is usually provided to reduce the risk of graft thrombosis, but does not improve graft survival [161]. For most pediatric patients with SLE, the overall survival post-transplant is comparable to that of children who receive a kidney transplant for other causes [16].

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Autoantibodies and Kidney Diseases

21

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Abstract

Autoimmune damage to the kidneys which can be triggered by various autoantibodies may cause a range of nephropathies. These autoantibodies may target specific renal structures like the glomerular basement membrane, resulting in primary diseases like anti-GBM disease, or they may be part of a systemic immune process such as vasculitis or systemic lupus erythematosus. There are also some other less common but serious systemic autoimmune conditions like antiphospholipid syndrome, which mainly causes thromboses via autoantibodies, may affect the kidneys, and causes devastating complications.

The evolving medical literature gives us more insight into even rarer autoimmune conditions affecting the renal system like cryoglobulinemic vasculitis or immunoglobulin G4-related disease. Although seen relatively rare in the pediatric population, autoantibody-related kidney damage may cause important morbidity, end-stage renal disease, and mortality. Recognition of these rare but important conditions is of paramount importance to make a correct diagnosis and introduce prompt treatment.

Keywords

Antiphospholipid · Autoantibody · Cryoglobulin · Glomerular basement membrane · Immunoglobulin G4

Synonyms

Goodpasture disease or syndrome; Non-anti-neutrophil cytoplasmic antibody (ANCA) associated small vessel vasculitis

Introduction

Autoimmune diseases affecting the kidney are relatively rare but can cause significant morbidity and mortality in the pediatric age. The target of autoantibodies may primarily be the kidney as anti-glomerular basement membrane (anti-GBM) disease and membranous nephropathy or renal damage may occur secondary to non-organ-specific autoantibodies such as systemic lupus erythematosus (SLE) and renal vasculitis.

The clinical presentation of autoimmune diseases with renal involvement is quite heterogeneous depending on the underlying disease.

Table 1 Examples of autoimmune diseases affecting the kidney and associated autoantibodies

Antiphospholipid syndrome	Lupus anticoagulant, anticardiolipin, anti-β2-glycoprotein-I antibodies
Cryoglobulinemia	Type I, II, III cryoglobulins
Immunoglobulin G4-related disease	Immunoglobulin G4
Anti-glomerular basement membrane disease	Anti-glomerular basement membrane antibodies
Systemic lupus erythematosus	Anti-dsDNA, antinucleosome autoantibodies
Renal vasculitis	Antineutrophil cytoplasmic antibodies
Primary membranous nephropathy	Anti-phospholipase A2 receptor antibodies, anti-thrombospondin type-1 domain-containing 7A antibodies
Atypical hemolytic uremic syndrome	Anti-complement factor H autoantibodies

Antiphospholipid syndrome (APS) may come to clinical attention with recurrent thrombosis or gestational problems in adolescent girls, whereas immunoglobulin G4-related disease (IgG4-RD) may present with autoimmune pancreatitis, cholangitis, lymphadenopathy, or orbital diseases. Kidney involvement widely ranged from thrombosis of renal vessels to glomerular diseases with the risk of end-stage renal disease.

Several autoantibodies have been shown to be associated with kidney involvement. A large variety of tests is now available to detect these autoantibodies which make early diagnosis and interventions possible (Table 1).

Antiphospholipid Syndrome

Antiphospholipid syndrome is a systemic autoimmune condition characterized by thrombosis and gestational morbidities associated with persistently positive pathogenic antiphospholipid antibodies (aPLs), including lupus anticoagulant (LA), anticardiolipin antibodies (aCL), and/or anti-β2-glycoprotein-I antibodies (aβ2-GPI) of immunoglobulin (Ig) G and/or IgM isotypes [66].

This condition is rare, and its precise incidence and prevalence in children are unknown. Pediatric APS refers to patients in whom the onset of APS is before 18 years of age. The criteria for diagnosis of pediatric APS are mainly based on adult Sapporo criteria, which are poorly validated in children [2]. The updated criteria used for diagnosis of APS in adult patients require at least one clinical event and one persistently positive aPL test (on two or more occasion at least 12 weeks apart) (Table 2) [66]. For pediatric APS generally, a positive aPL, on two or more occasions at least 12 weeks apart, in the setting of thrombosis is considered diagnostic. Although APS in children presents primarily as vascular thrombosis, less frequently it can present with isolated neurological and/or hematological disease.

APS may have diverse presentations like vascular events, gestational morbidity, catastrophic antiphospholipid syndrome (CAPS), asymptomatic aPLs, and non-classification criteria manifestations like autoimmune cytopenia, skin lesions, valvular heart disease, and APS nephropathy [57, 70]. Since the adult classification may lead to missed or delayed diagnoses in children, the “Single Hub and Access point for pediatric Rheumatology in Europe (SHARE) initiative” has developed recommendations for the diagnosis and treatment of childhood APS [39]. Besides the same laboratory criteria, non-thrombotic manifestations (hematologic, dermatologic, etc.) should be considered for the classification of pediatric patients.

In children 40–50% of APS is primary [5, 104]. Secondary APS constitutes around 50% of pediatric cases, most commonly due to SLE [5]. Primary APS tends to affect younger patients, and arterial thrombosis is more common. Patients with secondary APS, on the other hand, were older and show hematological and dermatological features and venous thrombosis [5].

Since there are overlapping features of SLE and APS, it is difficult to distinguish primary from

Table 2 Classification of antiphospholipid syndrome. (Updated Sapporo criteria. Adapted from Miyakis et al [66])

Clinical criteria	Vascular thrombosis	One or more episodes of arterial, venous, or small vessel thrombosis excluding superficial venous thrombosis
	Gestational morbidity	$\geq$1 unexplained deaths of a morphologically normal fetus $\geq$10 weeks' gestation
		$\geq$1 premature deliveries of a morphologically normal neonate <34 weeks' gestation
		Three or more consecutive spontaneous abortions before 10 weeks' gestation
Laboratory criteria	Two or more occasions, at least 12 weeks apart	Lupus anticoagulant (plasma)
		Medium- to high-titer anticardiolipin IgG and/or IgM antibody (serum or plasma)
		Titers >99th percentile of anti-β2-glycoprotein-I IgG and/or IgM antibody (serum or plasma)

secondary forms of APS. Almost 30% of children with primary APS show SLE or lupus-like disease during follow-up [5]. Patients with SLE might later develop APS. In a study on 57 children with SLE, 14% developed APS over 3-year follow-up [16]. Secondary APS has been seen in patients with juvenile idiopathic arthritis, but with rare thrombosis and less pathogenic aPLs [4]. Other diseases associated with secondary APS are IgA vasculitis, polyarteritis nodosa, Behçet disease, hemolytic uremic syndrome (HUS), and immune thrombocytopenic purpura [5, 67, 103].

Pathogenesis

The pathophysiology of APS is multifactorial and mainly results from the effects of aPLs on procoagulant factors and impaired fibrinolysis. For the development of antibodies, infectious triggers and rheumatological diseases have been proposed.

Antiphospholipid antibodies can be found in healthy children as well, but thrombosis is rare. The mechanism of generation of antibodies is unclear but might be due to molecular mimicry following infections or immunization. Low titers of aCL and aβ2-GPI antibodies were shown in 11.4% and 8.6% of healthy children, respectively [3]. Even infants may produce nonpathogenic anti-β2-GPI antibodies against antigens such as bovine β2-GPI found in milk or beef.

Antibodies in the disease process target not only phospholipids and phospholipid-binding proteins like β2-GPI but also cell-surface molecules such as annexin A2, apolipoprotein E receptor 2, toll-like receptors, interferon pathways, and the complement system (Fig. 1). The role of C4d in thrombotic microangiopathy (TMA) and SLE patients with positive aPLs was reported [88]. A recent study showed renovascular endothelial activation of mammalian target of rapamycin complex (mTORC) pathway in APS patients resulting in intimal hyperplasia [18].

Clinical Manifestations

Multiple organ systems are affected by APS. Table 3 depicts the clinical manifestations of the condition.

Catastrophic APS is a life-threatening complication of APS characterized by rapid microvascular thrombosis in multiple organs. Diagnostic criteria are the same as in adults [37]. Microangiopathic antiphospholipid-associated syndrome is defined as co-existence of aPLs and TMA in which the pathogenic role of aPLs is controversial. Pediatric HUS and SLE patients with microangiopathic antiphospholipid-associated syndrome have been reported [10, 61].

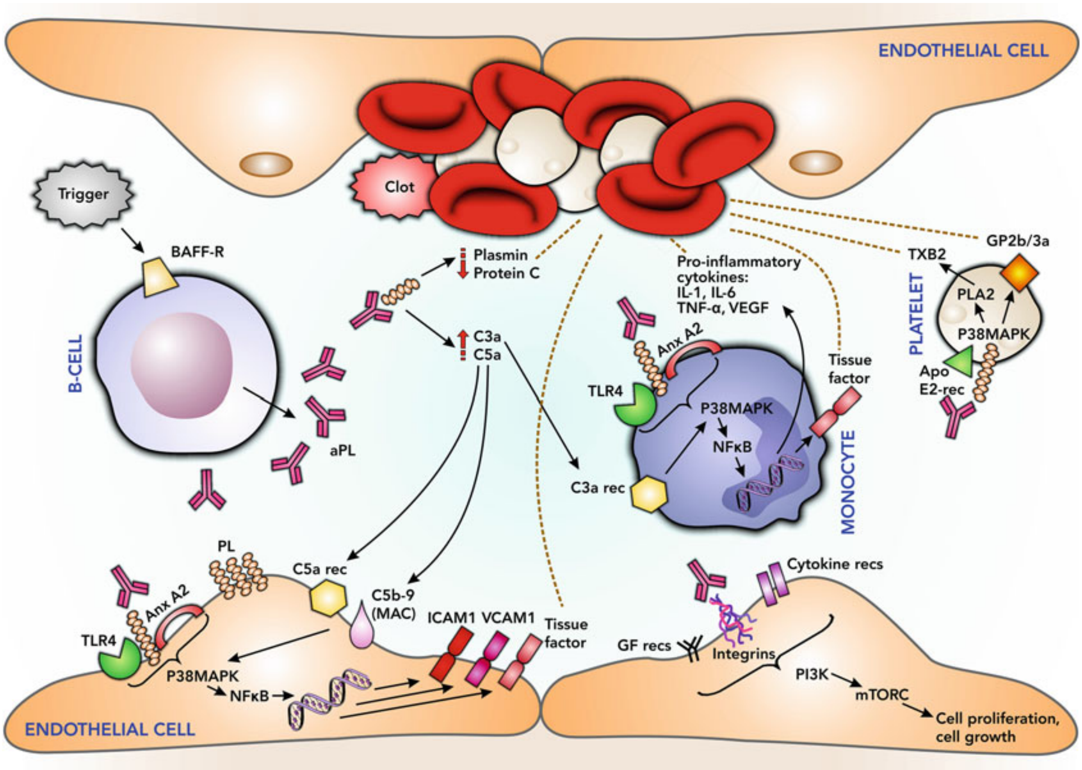
ENDOTHELIAL CELL
Trigger
Clot
BAFF-R
B-CELL
Plasmin
Protein C
C3a
C5a
aPL
Pro-inflammatory
cytokines:
IL-1, IL-6
TNF-α, VEGF
GP2b/3a
TXB2
PLA2
P38MAPK
PLATELET
Apo
E2-rec
Anx A2
TLR4
Tissue
factor
P38MAPK
NFκB
MONOCYTE
C3a rec
PL
C5a rec
C5b-9
(MAC)
ICAM1 VCAM1
Tissue
factor
Anx A2
TLR4
P38MAPK
NFκB
ENDOTHELIAL CELL
Cytokine recs
GF recs
Integrins
PI3K
mTORC
Cell proliferation,
cell growth

Fig. 1 Pathogenic mechanisms of antiphospholipid antibodies (aPL). (**a**) aPL-phospholipid (PL) complexes bind to disrupted endothelial surface and activate endothelial cell receptors, including toll-like receptors (TLR4) and annexin A2 (AnxA2) to promote activation of mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NFkB), and upregulation of tissue factor and adhesion molecules. (**b**) aPL-PL complexes also bind to apolipoprotein E receptor 2 (ApoE2-rec) on platelets and promote aggregation, via increased expression of glycoprotein 2b/3a (GP2b/3a) and thromboxane B2 (TXB2) synthesis. (**c**) aPLs impede plasmin and protein C pathways, inhibit anticoagulant proteins, and affect fibrinolysis. (**d**) On monocytes aPLs bind to AnxA2 and TLR4, increasing tissue factor expression and tumor necrosis factor-α (TNF-α) and cytokine production. (**e**) Complement activation may occur through downstream signaling. (**f**) aPL-PL complexes may also trigger vasculopathy by binding to endothelium and activating the mammalian target of rapamycin (mTOR) signaling. BAFF-R, B-cell activating factor receptor; C3a rec, complement 3a receptor; C5a rec, complement 5 a receptor; C5b-9 (MAC), membrane attack complex; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; PI3K, phosphatidylinositide 3′-kinase; VCAM-1, vascular cell adhesion molecule 1

Renal Involvement

While data is limited, the kidneys may be involved in both primary and secondary forms of APS. The disease may affect any part of the renal vasculature, from large vessels to glomerular capillaries. Renal artery thrombosis or stenosis, renal vein thrombosis, hypertension, APS nephropathy (APSN), chronic kidney disease (CKD), and renal allograft thrombosis may be seen.

The international Ped-APS registry consisting of 121 children reported 1 patient each with renal vein, renal artery, and mixed (artery and vein) thrombosis and 2 patients with renal thrombotic microangiopathy. Almost 35% of patients with secondary APS had lupus nephritis and 5% of patients with primary APS had renal involvement [5]. Ma et al. [57] reported 58 pediatric patients with APS, among which 40 had SLE-related APS with 65% (26/40) lupus

Table 3 Clinical manifestations associated with APS in children. (Adapted from Avcin et al [1, 5]

Thrombotic episodes
Venous
Extremities: deep venous thrombosis (most common); superficial venous thrombosis
Large vessels: Sinus vein thrombosis, superior/ inferior vena cava thrombosis
Pulmonary: pulmonary thromboembolism, pulmonary hypertension
Renal: renal vein thrombosis
Cardiac: left atrial thrombosis
Endocrine: Addison disease
Gastrointestinal: portal vein thrombosis, Budd-Chiari syndrome
Arterial
Cerebral: stroke, transient ischemic attack, acute ischemic encephalopathy
Extremities: peripheral arterial thrombosis
Renal: renal artery thrombosis
Cardiac: myocardial infarction
Abdomen: hepatic, splenic infarction
Small-vessel thrombosis
Digital ischemia; renal thrombotic microangiopathy
Non-thrombotic events
Hematologic
Thrombocytopenia, Evans syndrome, leukopenia, autoimmune hemolytic anemia
Bleeding diathesis (e.g., lupus anticoagulant-hypoprothrombinemia syndrome)
Dermatologic
Livedo reticularis, skin ulcers, chronic urticaria, Raynaud's phenomenon
Neurologic
Migraine headache; mood disorders; cognitive defects
Movement disorders
Pseudotumor cerebri
Ischemic stroke, seizures
Transverse myelitis; Guillain-Barre syndrome
Cardiologic
Valvular heart disease
Pulmonary
Pulmonary hypertension, interstitial fibrosis
Renal
Antiphospholipid nephropathy

nephritis. In the same study, there were two patients who experienced renal vein thrombosis. A study from North America on 22 children with APS reported no renal thrombotic events; of 8 patients with secondary APS, 3 had features of glomerulonephritis [70]. A Mexican study of 32 pediatric patients with APS did not reveal any renal manifestations [104].

Antiphospholipid Syndrome-Associated Nephropathy

The exact prevalence of APSN is not known. Performance of renal biopsies is a challenge to most clinicians due to bleeding risk related to accompanying thrombocytopenia and positive aPLs.

Nephropathy of APS may have different clinical presentations as nephritic or nephrotic syndrome and acute or chronic conditions. For years TMA has been the well-described pathology in biopsies of primary APS patients. Recent insights showed that other pathologies like fibrous intimal hyperplasia or focal cortical atrophy can also be seen. Membranous nephropathy and proliferative glomerulonephritis in primary APS are other examples [90]. In the Ped-APS registry, renal biopsies of three patients with secondary APS showed TMA findings [5]. Focal proliferative glomerulonephritis and pauci-immune necrotizing crescentic glomerulonephritis have also been described in pediatric primary APS patients [15, 65].

It should be kept in mind that TMA seen in APSN is a non-specific lesion, which can be seen in other diseases like thrombotic thrombocytopenic purpura and atypical HUS. Careful assessment is crucial to rule out these critical diagnoses.

Lupus Nephritis (LN) and aPLs

A cross-sectional study of children with SLE from the CARRA registry reported that the presence of aPLs significantly increased the thrombosis risk [30]. A prospective study in 56 children with SLE revealed that aPL-positive children had three times higher risk of organ damage [28]. Another study on 150 SLE patients (44 patients <15 years of age) found that 34% of the patients had APSN and APSN was associated with HT, acute renal failure, persistent proteinuria, severe LN histology (Class 3 and 4), and end-stage renal disease (ESRD) [21].

Catastrophic APS and Kidneys

The pediatric CAPS registry including 46 patients showed 63% of patients had kidney manifestations mainly as renal failure, arterial HT, and proteinuria. Compared to adults, prevalence of renal involvement wasn't significantly different in children (74.2 vs. 63%) [9].

Tektonidou et al. evaluated biopsies of APSN patients and found acute lesions to be more common in CAPS than in primary APS and SLE-APS; chronic lesions like fibrous intimal hyperplasia and focal cortical atrophy were found in similar frequencies among the three groups [97].

Antiphospholipid Syndrome and End-Stage Renal Disease (ESRD)

Little is known about aPLs and overall risk of CKD. In adults CKD incidence ranged between 28–60% in primary and 16–54% in SLE-related APS [27]. In children there are few anecdotal case reports on CKD and ESRD associated with APS [15, 73]. The Ped-APS registry and APS series from different parts of the world did not report any ESRD among their study populations [5, 57, 70, 104]. New studies with broader timescales to explore the long-term renal outcomes are needed.

Among adult patients on hemodialysis (HD), aPL prevalence was found to be high and associated with thrombosis [14]. Renal transplantation in APS carries a high-risk for TMA development, often quite severe. Evidence suggests patients with aPLs have increased risk of early graft loss due to thrombosis of graft vessels or TMA [17]. A recent study on transplant patients showed aCLs increased the risk of estimated glomerular filtration rate decline in 1st year posttransplantation [34].

Treatment

There is limited data on the optimal treatment for pediatric APS. Most of our knowledge is extrapolated from adult studies, and recommendations are mainly expert opinions. The main aims are preventing, treating thrombosis, and preventing recurrence. Treating the underlying disease in secondary APS forms and balancing the potential side effects of therapeutics are other important goals. In healthy children with aPLs, antibodies are usually low-titer, transient, and clinically insignificant; therefore no treatment or prophylaxis is necessary (Table 4).

Children with autoimmune diseases have chronic inflammation as an extra pro-thrombotic risk; therefore the SHARE initiative suggested use of anti-platelet dose aspirin in addition to hydroxychloroquine in children with aPL(+) SLE [39].

Treatment of acute thrombosis in APS is the same as in other diseases and needs consultation with pediatric hematology. A loading dose of unfractionated heparin, followed by its maintenance, is the traditional method. Due to ease of administration, predictable dose-response, and less need for monitoring, low molecular weight heparin (LMWH) is also being considered [58]. After a treatment period with LMWH, children are transitioned to warfarin. In high-risk thrombosis cases, thrombolytic therapy should be cautiously administered. For children, bleeding risks during play and sports should be sought while treatment planning.

Currently new agents with less need for monitoring and side effects are being tested. Direct

Table 4 Recommendations for thromboprophylaxis in children with aPLs/APS

1. Primary thromboprophylaxis
In asymptomatic patients with aPL positivity:
Control of pro-thrombotic risks; smoking, obesity, hyperlipidemia, estrogen-containing oral contraceptives.
In patients with SLE and positive aPLs:
Hydroxychloroquine and anti-aggregant dose aspirin [39]
2. Secondary thromboprophylaxis
No treatment when aPLs disappear [39]
Continue warfarin in persistent aPL positivity, target INR:2-3 [32], target higher INR, or consider other therapeutics if thrombosis recurs [39]
Add aspirin to anticoagulant therapy when history of arterial thrombosis [39]

Note: aPL, antiphospholipid antibody; APS, antiphospholipid syndrome; INR, international normalized ratio; SLE, systemic lupus erythematosus thrombin inhibitors (dabigatran), factor Xa inhibitors (apixaban, rivaroxaban), non-warfarin/heparin anticoagulants, hydroxychloroquine, statins, B-cell inhibitors, complement inhibitors, and peptide therapies are some examples. An adult study compared rivaroxaban and warfarin in APS patients with/without SLE and showed this new agent could be a safe alternative in APS patients with venous thrombosis history [23]. Subsequent study comparing rivaroxaban and warfarin in high-risk patients with APS showed significantly higher rate of thromboembolic events in the rivaroxaban arm and consequently, oral anticoagulants are not recommended in APS patients with triple aPL positivity and arterial thrombosis [76, 98].

Treatment of CAPS

There aren't any pediatric guidelines for CAPS treatment. The literature emphasizes the importance of prompt and aggressive treatment. General approach involves anticoagulation for TMA and immunosuppression to control the cytokine storm. The SHARE initiative recommended prompt use of combined anticoagulants, steroids, and plasma exchange with/without intravenous Ig [39]. Treatment of inciting factors, most commonly the infections, is of critical importance.

Rituximab may also be considered. In the CAPS registry, 20 patients were treated with rituximab as first- or second-line therapy and the majority benefited [8]. Eculizumab was also effectively used for CAPS [106].

Treatment of APSN

There is no consensus on the optimal treatment of APSN. In general it is the same as for APS. Patients with APSN who meet the APS criteria should receive the standard anticoagulatory treatment. In adult APSN with SLE hydroxychloroquine and immunosuppressive treatment is advised [96]. In APS patients with membranous nephropathy, prophylactic anticoagulation should be given due to high thrombosis risk. Hypertension and proteinuria are commonly seen in APSN; therefore angiotensin-converting enzyme inhibitors should be considered. The use of novel oral anticoagulants has not been studied in APSN.

Eculizumab has been successfully used in the posttransplant period of aPL-positive patients with recurrent TMA [40]. Studies showed that rituximab had some effect on renal lesions [31]. Sirolimus (mTOR inhibitor) in transplant patients resulted in longer survival in graft function via decreasing vascular cellular proliferation, inhibition of fibrous intimal hyperplasia, and chronic lesions [18]. However long-term safety data are needed for both agents.

In HD patients with aPLs, anticoagulants may increase the arteriovenous graft survival; however taking the bleeding risk into account, it is advisable to anticoagulate only patients with recurrent graft thrombosis. Perioperative anticoagulation during transplantation may protect from graft failure in patients with aPLs or APS; however thrombosis might still occur.

Anti-glomerular Basement Membrane Disease

Definition

Anti-glomerular basement membrane (anti-GBM) disease is an autoimmune disease in which autoantibodies directed against the noncollagenous-1 (NC1) domain of the α3-chain of collagen type IV affect the kidneys and lungs, resulting in rapidly progressive glomerulonephritis and/or pulmonary disease [7, 38]. Goodpasture syndrome and Goodpasture disease are older terms used synonymously for anti-GBM disease, but also for the clinical constellation of glomerulonephritis and pulmonary hemorrhage (pulmonary renal syndrome), regardless of the underlying pathogenesis.

Epidemiology

Anti-GBM disease is uncommon in adults and extremely rare in children. It is estimated to occur in less than two cases per million population, even less in children – one case per two million population [19, 59]. It accounts for 1–5% of all types of glomerulonephritis and 10–20% of crescentic glomerulonephritis but is a rare cause of end-stage renal disease (ESRD) [49, 95].

In adults, the first peak occurs in young men aged 20–30 years, and the second one in those aged 50–70 years, with men and women equally affected. In children, anti-GBM disease has been reported in all ages, with a peak incidence at 17 years of age [12, 100].

Etiology

Anti-GBM disease results from environmental insults in a person with a genetic predisposition. An exact trigger has not been identified for the development of anti-GBM antibodies, but when they do develop, circulating antibodies can cause renal and/or pulmonary disease. These are usually of the IgG class, with predominant IgG1 and IgG3 subclasses [87]. They are typically polyclonal, but rare cases of monoclonal antibody derived anti-GBM disease have also been described [75]. Antibodies against the α3-chain of collagen type IV can also be detected in healthy individuals, but these have a different IgG subclass distribution, with lower titer and avidity, probably representing natural autoantibodies [24].

An increased genetic susceptibility to Goodpasture syndrome can be seen in humans with certain human leukocyte antigen (HLA) subtypes, most notably HLA-DR15 and HLA-DR4 [77]. While the expression of the α3-chain of collagen type IV is highest in the glomerular and alveolar basement membrane, these antibodies predominantly cause symptoms derived from these two organ systems. However, not all patients with renal also develop pulmonary symptoms and vice versa, even though they all appear to have similar autoantibodies against the α3-chain of collagen type IV [44]. It seems that prior pulmonary and kidney injury/triggers are needed to “expose” the antigens in the glomerular and alveolar basement membranes. Possible triggers of anti-GBM disease, such as pulmonary infections, smoking, hydrocarbon solvents, lung cancer, lithotripsy, ureteral obstruction, membranous nephropathy, and ANCA-associated glomerulonephritis, have been described. In addition, immune dysregulation, namely, hematopoietic cell transplantation, alemtuzumab, checkpoint inhibitors, T-cell large granular lymphocyte leukemia, common variable immunodeficiency, and HIV, also appear to predispose to anti-GBM antibody generation and anti-GBM disease [80].

Autoreactive T-cells, in addition to enhancing B-cell function and antibody production, may have a direct causative role in glomerular and alveolar injury. It was shown that T-cells isolated from patients with anti-GBM disease, but not from controls, react against the α3-chain of collagen type IV and play a role in crescent formation [64].

Clinical Presentation

Most patients with anti-GBM disease present with systemic complaints and clinical features of renal and/or pulmonary disease; other organ involvement is also possible.

Renal manifestations can vary widely, from hematuria and proteinuria to a clinical picture of rapidly progressive glomerulonephritis with rapidly progressive renal failure, oliguria, volume overload, edema, and severe hypertension.

Pulmonary symptoms may or may not precede renal symptoms by weeks or months and appear most commonly with hemoptysis, which can be so profound as to cause respiratory failure and death, or can only be occult and lead to anemia and iron deficiency.

Systemic complaints and signs can be the initial presentation of anti-GBM and consist of symptoms such as malaise, weight loss, fever, and arthralgia. These are usually experienced for only a few weeks, and when present for a longer period, suggest that a patient is double positive for anti-GBM and antimyeloperoxidase (MPO-ANCA) and has features of concurrent vasculitis [26, 49, 80].

Other organ involvement is rare, but not exclusive. A case report of a teenager with cerebral vasculitis with confusion, aphasia, and seizures has been reported [36].

Double-positive anti-GBM and ANCA-associated disease has been described in 10–50 percent of patients with anti-GBM disease [53]. These patients tend to have a higher rate of relapses, a generally worse pattern of disease and the need for early treatment in order to achieve a better outcome [60], and poorer recovery of renal function, potentially linked to greater glomerular damage due to the presence of both antibodies [53].

There is up to 50 percent of recurrent linear IgG staining in the transplant, but most patients remain asymptomatic [50, 59]. The target GBM antigens for recurrent anti-GBM disease are the noncollagenous-1 (NC1) domain of the α3- and α5-chains of collagen type IV. Similar findings can be found in Alport posttransplant anti-GBM nephritis, where anti-GBM syndrome is mediated by the deposition of alloantibodies to the α5-chains of collagen type IV in response to the "foreign-transplanted" collagen antigen normally absent in patients with Alport syndrome but present in the renal allograft [42]. Most patients remain asymptomatic, while patients with clinically evident recurrence of anti-GBM disease after transplantation can have a wide range of symptoms, the onset of which can range from months to years posttransplant.

Diagnosis

Anti-GBM disease should be suspected in any patient with acute/subacute nephritic syndrome, particularly if it is rapidly progressive and/or accompanied by alveolar hemorrhage. It should also be suspected in a patient with isolated alveolar hemorrhage; patients with features of systemic disease; patients with nephrotic syndrome who develop a rapid decline of renal function, patients with proteinuria, hematuria, or mild renal insufficiency with linear IgG staining of the GBM on kidney biopsy; and kidney transplant recipients with underlying Alport syndrome who present with acute glomerulonephritis or graft failure. The diagnosis is thereafter confirmed by the demonstration of anti-GBM antibodies in the serum or the kidney/lung [49]. If not contraindicated, renal biopsy should be performed, since the accuracy of serological testing is variable and, in addition, it provides additional and important information regarding the activity and chronicity of renal involvement that may help guide therapy.

In general, a direct enzyme-linked immunoassay (ELISA) with purified or semi-purified Goodpasture syndrome antigens is the most commonly used assay. False-positive and false-negative results may occasionally occur [55]. Sensitivity ranges from 63–100% and depends on the commercial kit used.

Western blot, considered as a reference test, is used in some cases to confirm the antigenic (NC1 – domain of the α3-chain of collagen type IV) specificity of anti-GBM antibodies [49].

Indirect immunofluorescence is a rarely performed historical test that requires an experienced renal pathologist. It is performed by incubating normal renal tissue with the patient's serum and then treating it with fluorescein-labeled anti-IgG. The test has a high false-negative rate, and the difficulty in detecting a low titer of anti-GBM antibodies explains why this technique is no longer recommended [80].

In addition to anti-GBM antibody testing, testing for antineutrophil cytoplasmic antibodies (ANCA) should be performed in all patients who are diagnosed with anti-GBM disease, since the clinical course and treatment plan may differ in double-positive patients [7, 53].

As neither method for anti-GBM antibody detection is completely reliable, a kidney biopsy is always recommended unless contraindicated. Light microscopy most commonly shows crescentic glomerulonephritis, while a virtually pathognomonic finding of bright linear IgG deposits along GBM is seen by immunofluorescence. In rare cases, the antibody may be IgA or IgM [6].

Pulmonary biopsy in not necessary when a kidney biopsy and serological tests have already confirmed the diagnosis of anti-GBM disease but can be done in an isolated pulmonary disease. Pulmonary histopathology often shows a linear deposition of IgG, but this finding is not as constant as in a kidney biopsy [80].

Differential Diagnosis

Other causes of acute glomerulonephritis associated with pulmonary hemorrhage (pulmonary renal syndromes) and other renal disorders in which linear IgG staining may be seen in the glomeruli on a kidney biopsy should be considered in the differential diagnosis of anti-GBM disease.

Pulmonary hemorrhage can be seen with other acute glomerulonephritis when complicated by pulmonary edema or lung involvement in systemic vasculitis (ANCA-positive, IgA vasculitis, and mixed cryoglobulinemia syndrome) and systemic lupus erythematosus. Antiphospholipid syndrome and pulmonary infection with concomitant acute kidney injury may also present, for example, pulmonary renal syndromes. Differentiation between these disorders is difficult on the basis of a clinical picture alone, and serological testing and kidney biopsy are required to establish the diagnosis.

Linear IgG staining on kidney biopsy may be seen in diabetic nephropathy and fibrillary glomerulonephritis, though staining is rarely as strong as with anti-GBM disease, and there are several other issues on the basis of which these two entities can be easily distinguished from anti-GBM disease (circulating anti-GBM antibodies are absent in both disorders, crescents are not seen in diabetic nephropathy, characteristic fibrils are seen on electron microscopy in fibrillary glomerulonephritis) [80].

Treatment

Recommendations for the treatment of anti-GBM disease in children are mainly based on studies in adults. Early diagnosis is vital in terms of ability to recover renal function [79, 80]. In addition to early diagnosis, the degree of initial renal impairment is also a very important prognostic factor. It was reported that avoidance of end-stage renal disease is very rare in patients who require dialysis within 72 h, in particular if they have crescents involving all glomeruli. On the other hand, prevention of end-stage renal disease can usually be achieved in less severe cases, although some do progress [52]. KDIGO adult guidelines therefore recommend avoiding immunosuppressive treatment in patients who are dialysis-dependent on presentation or who have 100% crescents in an adequate biopsy sample, in the absence of pulmonary hemorrhage [72]. Nevertheless, more than 50% of nephrology centers do not follow this recommendation and still treat these patients [52, 53, 63]. The inability to consistently and accurately identify the dialysis-dependent patient who may recover renal function has led some investigators to nevertheless treat patients with very acute disease, young patients who can tolerate aggressive immunosuppression better, and patients with anti-GBM antibody disease who are also double-positive for ANCA and have clinical signs of systemic vasculitis [53].

The treatment of choice in anti-GBM disease is intensive plasmapheresis [52, 56, 79]. Plasmapheresis is aimed to remove circulating antibodies, while the purpose of immunosuppression is to reduce their production. Plasmapheresis must be accompanied by aggressive immunosuppression consisting of corticosteroids and cyclophosphamide [25]. An alternative, rituximab or mycophenolate mofetil, has also been tried [68, 94].

The optimal duration of therapy is unknown. Anti-GBM antibody titers are monitored regularly (every 1–2 weeks until they are negative on two occasions), and treatment with plasmapheresis is stopped or prolonged depending on their levels and the patient's clinical condition [79]. Once a remission of the disease is achieved, maintenance with low-dose prednisone and azathioprine should be continued for 6–9 months.

A rather novel approach in treating anti-GBM disease is immunoadsorption, which can remove pathogenic IgG from circulation and does not require plasma infusions as in plasmapheresis; it may be/prove effective in anti-GBM disease [11, 29].

Experimentally, the development of crescentic glomerulonephritis was completely prevented in a rat model of anti-GBM antibody disease, with a blockade of CD28-B7, a costimulatory pathway for T-cell activation, by the fusion protein CTLA4Ig [82].

Outcome

Before the era of immunosuppressives such as corticosteroids and before the era of plasmapheresis, most patients with anti-GBM disease died of pulmonary hemorrhage or renal failure. The current therapeutic approach has reduced mortality among adults to less than 20% and in children to around 30% [78, 86].

Outcome seems to be directly correlated with initial renal impairment and the percentage of crescents on the initial kidney biopsy specimen. Despite potentially serious lung hemorrhage, there is virtually no residual pulmonary deficit or fibrosis when patients recover [49]. Recurrence of the disease with antibody production is possible, but rare, as it occurs spontaneously or is precipitated by infection or exposure to a toxic agent [49, 62]. Renal transplantation can initiate antibody production [48, 50]. Patients with anti-GBM and ANCA antibodies present with a better renal outcome but may experience more relapses [53].

IgG4-Related Disease

Introduction

IgG4-related disease (IgG4-RD) is a multi-organ fibro-inflammatory disease, including autoimmune pancreatitis (AIP), Mikulicz' disease, sclerosing cholangitis, respiratory disease, thyroid disease, lymphadenopathy, periaortitis, retroperitoneal fibrosis, and kidney disease [92]. Hamano et al. were the first to note elevation of serum IgG4 levels among patients with AIP, in concert with predominant infiltration of IgG4-bearing plasma cells in the pancreatic tissue [41]. Subsequently, Kamisawa et al. demonstrated increases of IgG4-bearing plasma cells in a variety of organs in patients with AIP and proposed the concept of IgG4-related autoimmune disease [45].

The prevalence of IgG-RD has been unclear, because this disease entity was seldom recognized until recently and integrated data on the involvement of different organs is absent. IgG4-RD predominantly occurs in elderly males (male-female=2:1, 58.2 ± 14.5 years), and reports of pediatric cases were rare. A systematic literature search found 25 cases of IgG4-RD in pediatric patients, and the youngest one was a 3-year-old female with cholangitis [46]. The review showed female predominance, and orbital disease was associated in about half of the pediatric patients. Comprehensive view of IgG4-RD as a systemic disease has been accepted among different specialties, and several important collaborative works have been reported [93, 101].

The clinical features of IgG4-RD are varied, depending on the affected organs and the degree of activity and stage of each organ's involvement. IgG4-RD could be divided into four clinical features, including pancreato-hepato-biliary disease, retroperitoneal fibrosis and/or aortitis, head and neck-limited disease, and classic Mikulicz syndrome with systemic involvement. Organ involvement might not occur simultaneously, and it often appears in a metachronous manner.

Is IgG4-RD an Autoimmune Disease?

IgG4-RD has been considered as an immune-mediated disease, because AIP has several immune-related features, i.e., hypergammaglobulinemia, presence of various autoantibodies, lymphoplasmacytoid infiltration, and effectiveness of steroid [84]. Immunological characteristics, typically oligoclonal expansion of IgG4-positive plasmablasts, appear in the circulation and the tissue, and circulating plasmablasts and activated B cells frequently revealed somatic hypermutation, indicating an adaptive immune response driven by an antigen in IgG4-RD. In addition, recent report revealed activation of T follicular helper cell (Tfh) and synthesis of Th2 cytokines in IgG4-RD, suggesting autoimmune pathogenesis [51]. Alternative pathogenesis of IgG4-RD may be allergy, because of Th2 activation [105]. Indeed, one third of IgG-RD patients have a history of allergic diseases, such as asthma, rhinitis and atopic dermatitis, and hyperproduction of IgG4 or eosinophilia. The mechanism of IgG4-RD may be based on the complex immune-related responses. Typically, a direct role of IgG4 and/or IgG4-bearing cells in tissue damage and the roles of lymphocyte activation linking to tissue fibrosis have not been identified.

IgG4-Related Kidney Disease

IgG4-related kidney disease (IgG4-RKD) reveals characteristic clinical, radiological, and pathological features, being the main basis of its diagnosis.

Clinical Features

Like IgG4-RD in general, IgG4-RKD frequently occurs in elderly males. The mean age was 64 years, and 73% of the patients were male [47]. Pediatric cases with IgG4-RKD were very rare and only a few patients were reported. Unfortunately, detailed description as IgG4-RKD has not been given in the literatures [46]. Either elevation of serum IgG levels, hypocomplementemia, or high IgE levels is associated with IgG4-RKD. The clinical features of IgG4-RKD largely reflect the characteristic tubulointerstitial nephritis (TIN). The onset of nephropathy is rather insidious, and the disease can often be identified as a subacute or chronic renal dysfunction. The levels of proteinuria are generally mild and hematuria is at minimal levels. As is typical in chronic TIN, β2-microglobulin and N-acetyl-β-D-glucosaminidase are useful for the diagnosis of IgG4-RKD. In cases with nephrotic syndrome, albeit rare, it may be membranous nephropathy or fortuitous association of other kidney diseases such as minimal change disease. IgG4-RKD is often progressive and leads to chronic renal failure, if the response to therapy is poor. On the other hand, there are cases with spontaneous remission.

Radiological Findings

Radiological examination by contrast-enhanced computed tomography (CT) and magnetic resonance imaging has great value for the diagnosis. Radiological findings reveal various features, i.e., bilateral kidney swelling, poorly enhanced wedge- or round-shaped mass, or multiple nodular lesions (Fig. 2a). A single space-occupying shadow has been misdiagnosed as kidney tumor and surgically removed.

Kidney Pathology

Kidney pathology is an important diagnostic basis and provides clues to understand the pathophysiology of progressive IgG4-RKD. It reveals characteristic tubulointerstitial nephritis as follows (Fig. 2b–f): (1) clear border with adjacent preserved parenchyma (Fig. 2b); (2) plasma cell-rich infiltration with predominant IgG4-bearing cells (Fig. 2c, e, f); (3) lack of neutrophil infiltration and tubulitis; (4) fibro-inflammatory changes in the cortex, the medulla, and the renal capsule; (5) a characteristic pattern of interstitial fibrosis, called “bird’s eye pattern” or “storiform fibrosis” (Fig. 2d); and (6) immune deposition along the tubular basement membrane, IgG4 and/or IgG1 and C3 and C1q [102]. Sjögren’s syndrome, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, and interstitial lupus nephritis are similar to IgG4-RKD and need discrimination. Membranous nephropathy (MN) is another kidney disease associated with IgG4-RD, in patients with nephrotic syndrome. Immunofluorescence reveals a granular pattern of IgG4 deposition along the glomerular basement membrane (GBM). Anti-M-type phospholipase A2 receptor antibody (antigen of idiopathic MN) is useful to discriminate IgG4-related MN and IgG4-RD with idiopathic MN.

Diagnostic Criteria

Diagnosis of IgG4-RKD is not always easy, because of clinical and pathological heterogeneity and because the diagnosis is made by several inclusion criteria. The American College of Rheumatology/European League Against Rheumatism classification criteria for IgG4-RD recommended the following inclusion criteria for IgG4-RKD [101]: (1) hypocomplementemia with low serum levels of C3, C4, or both; (2) renal pelvic wall thickening, either unilateral or bilateral, usually without severe stenosis or luminal irregularity; and (3) low-density areas in both renal cortices seen only on contrast-enhanced CT and usually patchy or round-shaped in appearance. The diagnostic criteria of IgG4-RKD in 2020 had a sensitivity of 90.9% and specificity of 90% (Table 5) [85]. It should be noted that ANCA is often positive in cases with IgG4-RD,

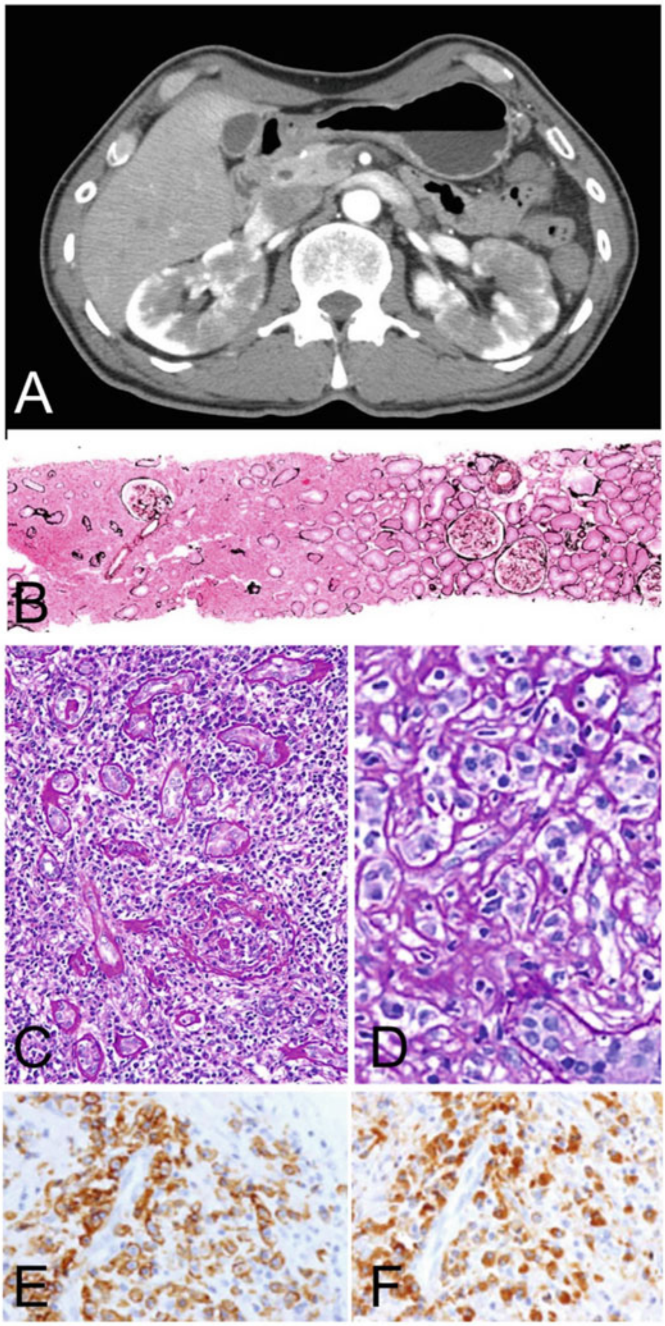

Fig. 2 Radiological and histological characteristics of IgG4-RKD. (**a**) Contrast-enhanced computed tomography showing multiple poorly enhanced lesions in the bilateral kidney. (**b**) Kidney biopsy sample with PAM stain reveals that tubular loss with intensive inflammatory infiltrates is clearly demarcated by the neighboring preserved renal parenchyma. (**c**) Larger magnification view of renal cortex showing plasma cell-predominant infiltration. (**d**) Characteristic pattern of tubulointerstitial fibrosis, called "bird's eye" or "storiform" pattern of fibrosis (**e**). Immunohistochemistry of IgG (**e**) and IgG4 (**f**). Note that IgG4-bearing cells are the major component of IgG-positive plasma cells. (**a**, **b**, **e**, and **f** were from reference 4 with permission)

and eosinophilic granulomatous polyangiitis and granulomatosis with polyangiitis occasionally fulfill the criteria for IgG-RKD.

Treatment

Good response to steroids is a distinct characteristic of IgG4-RD that helps the diagnosis. Steroids

Table 5 Diagnostic criteria for IgG4-related kidney disease (IgG4-RKD) 2020

1. Presence of some kidney damage; abnormal urinalysis or urine marker(s) or decreased kidney function with either elevated serum IgG level, hypocomplementemia, or elevated serum IgE level
2. Abnormal renal radiological findings:
(a) Multiple low-density lesions on enhanced computed tomography
(b) Diffuse kidney enlargement
(c) Hypovascular solitary mass in the kidney
(d) Hypertrophic lesion of renal pelvic wall without irregularity of the renal pelvic surface
3. Elevated serum IgG4 level (IgG4 > 135 mg/dl)
4. Histologic findings in the kidney
(a) Dense lymphoplasmacytic infiltration with infiltrating IgG4-positive plasma cells >10/high power field (HPF) and/or IgG4/IgG-positive plasma cells >40%
(b) Characteristic fibrosis surrounding nests of lymphocytes and/or plasma cells
5. Extra-renal organ(s):
(a) Dense lymphoplasmacytic infiltration with infiltrating IgG4-positive plasma cells >10/HPF and IgG4/IgG-positive plasma cells >40% in extra-renal organ(s)
(b) Imaging or clinical findings in extra-renal organ(s); one of the following items;
1. Bilateral lacrimal gland swelling
2. Bilateral submandibular or parotid gland swelling
3. Imaging findings compatible with type 1 autoimmune pancreatitis
4. Imaging features of retroperitoneal fibrosis

Definite: Any of the below combinations
1 + 3 + 4a + 4b; 2 + 3 + 4a + 4b; 2 + 3 + 5a; 1 + 3 + 4a + 5a or 5b; 2 + 3 + 4a + 5b
Probable: Any of the below combinations
1 + 4a + 4b; 2 + 4a + 4b; 2 + 5a; 2 + 3 + 5b
Possible: Any of the below combinations
1 + 3; 2 + 3; 1 + 4a; 2 + 4a; 2 + 5b
Appendix 1. Clinically and histologically, exclusion of the following diseases should be considered: ANCA-associated vasculitis, multicentric Castleman's disease, malignant lymphoma, and extramedullary plasmacytoma. 2. Radiologically, exclusion of the following diseases should be considered: malignant lymphoma, urinary tract carcinoma, renal infarction, and pyelonephritis (rarely, granulomatosis with polyangiitis, sarcoidosis, and metastatic carcinoma)

are also known to be effective in IgG4-RKD, being the first choice, and early steroid immediately after the diagnosis is important in the treatment of IgG4-RKD. Although initial effects of steroid are favorable, tapering or stopping steroid often induces recurrence. Hypocomplementemia and high serum IgG4 are paralleled with steroid treatment, being useful markers for therapeutic effects. Several reports of IgG4-RD described usage of azathioprine, tacrolimus, mycophenolate mofetil, and abatacept in the cases with unfavorable response to the steroid; however, their safety and effectiveness have not been well established.

Cryoglobulinemic Vasculitis

Cryoglobulinemic vasculitis is an immune complex-mediated inflammation of the small- and medium-sized blood vessels, leading to skin and internal organ involvement, including the kidney. It is characterized by the presence of cryoglobulins, which are immunoglobulins that precipitate when the temperature falls below 37 °C and re-solubilize on warming. Cryoglobulinemic vasculitis rarely occurs in isolated, primary form and is usually associated with other diseases such as infections, autoimmune diseases, and lymphoproliferative disorders [83].

Definition and Classification

Cryoglobulinemia refers to the presence of cryoglobulins in serum. Based on the Brouet classification [13], three major groups of cryoglobulins are recognized according to the clonality and type of immunoglobulins:

- Type I cryoglobulins consist of single, monoclonal immunoglobulin, usually IgM and sometimes IgG or IgA
- Type II cryoglobulins are a mixture of monoclonal IgM and polyclonal IgG; the monoclonal IgM usually have rheumatoid factor activity
- Type III cryoglobulins are a mixture of polyclonal IgM and IgG

Type I cryoglobulinemia is associated exclusively with B-cell proliferative diseases. Type II and III cryoglobulinemias are classified as "mixed cryoglobulinemias" because they consist of both IgG and IgM components. Both type II and type III mixed cryoglobulinemias are associated with hepatitis C virus (HCV) infection, autoimmune diseases, or B-cell lymphoproliferative diseases.

The presence of cryoglobulins can be detectable in the serum of patients with several inflammatory conditions, but it remains asymptomatic in most of them. The term "cryoglobulinemic vasculitis" is used to define the presence of cryoglobulins and the clinically apparent disorder with cutaneous vasculitis, nephritis, and other organ involvement [81]. According to the 2012 International Chapel Hill consensus nomenclature, cryoglobulinemic vasculitis is included among the non-antineutrophil cytoplasmic antibody (ANCA)-associated small vessel vasculitides [43].

Cryofibrinogenemia is a rare form of cryopathy defined by the presence of a cold-induced precipitate found in plasma, which is characteristically composed of fibrinogen, fibrin, fibronectin, and fibrin degradation products [22].

Epidemiology

Cryoglobulinemic vasculitis is a rare disease with less than 5 cases per 10,000 individuals, but the prevalence is significantly higher in some geographical regions such as the Mediterranean Basin. The most common cause is chronic infection with HCV which globally affects more than 184 million individuals. Mixed cryoglobulinemia was detected in up to 60% of patients with HCV infection, but only 5% of individuals with HCV infection develop cryoglobulinemic vasculitis. About 10–20% of cases of mixed cryoglobulinemia are not associated with HCV infection and are associated with other infections (hepatitis B virus, HIV, bacterial endocarditis), autoimmune diseases (Sjögren's syndrome, systemic lupus erythematosus, dermatomyositis), and malignancies (B-cell lymphoproliferative diseases). Essential or idiopathic cryoglobulinemia represents approximately 10% of cases of mixed cryoglobulinemia [83]. The frequency of cryoglobulinemia in pediatric population represents about 15–20% of adult cases [54].

Etiopathogenesis

Cryoglobulins are generated by persistent immune stimulation caused by chronic infections or autoimmune diseases and in the case of malignancies by the clonal expansion of B cells. HCV is able to infect both hepatocytes and B cells causing polyclonal B-cell proliferation as well as B-cell transformation with predominantly IgM-producing memory B cells and increased expression of lymphomagenesis-related genes critical for somatic hypermutation and prevention of B-cell apoptosis. Chronic antigenic stimulation by HCV infection leads to widespread production of heterogeneous autoantibodies.

Precipitation of cryoglobulins could be triggered by cold exposures and in the case of mixed cryoglobulinemia by the formation of large, complement-bound immune complexes formed by circulating HCV particles, anti-HCV polyclonal IgG, and monoclonal IgM with rheumatoid factor activity. Tissue injury in cryoglobulinemic vasculitis is caused by immune complex-mediated inflammation in small blood vessels and vascular occlusion due to cryoglobulin precipitation in the microcirculation. In cryoglobulinemic glomerulonephritis, there is additional macrophage recruitment to the glomerulus trying to remove deposited cryoglobulins. Ineffective cryoglobulin clearance by phagocytes could further perpetuate glomerular damage [91].

Familial cases of cryofibrinogenemia were found in patients with *TREX1* gene mutation most of whom presented in the childhood age with cold-induced acrocyanosis [74].

Clinical Manifestations

Clinical manifestations of cryoglobulinemia are caused by two principal mechanisms. The first mechanism is hyperviscosity and occlusion of small blood vessels caused by cryoglobulin precipitation. Common manifestations in patients with hyperviscosity syndrome include Raynaud's phenomenon, cold-induced acral ulcers, digital gangrene, livedo reticularis, retinal hemorrhages, blurry vision, epistaxis, headache, confusion, and encephalopathy. These manifestations are common in type I cryoglobulinemia with high cryoglobulin concentrations [81].

The second mechanism is immune complex-mediated vasculitis of small blood vessels, which is common in types II and III mixed cryoglobulinemias. The most common manifestations of cryoglobulinemic vasculitis are cutaneous purpura, arthralgia, arthritis, kidney involvement, and peripheral neuropathy. Flares of cryoglobulinemic vasculitis are often accompanied also by general symptoms such as fever, weakness, and myalgia [81]. Kidney involvement usually displays as isolated proteinuria or hematuria and less commonly as nephritic syndrome or acute kidney failure [71]. Children with cryoglobulinemia more commonly present with fever and musculoskeletal and cutaneous involvement [54].

Diagnosis

The diagnosis of cryoglobulinemic vasculitis requires demonstration of cryoglobulins in association with suggestive clinical vasculitis symptoms. Decreased serum levels of early components of the complement cascade (C4) and raised titers of rheumatoid factor are commonly seen in mixed cryoglobulinemias. The presence of urinary abnormalities (hematuria, proteinuria) and hypertension demonstrate renal involvement and require confirmation by renal biopsy. Renal biopsy usually shows type I membranoproliferative glomerulonephritis with subendothelial IgG, IgM, and C3 deposits, hyaline intraluminal thrombi, and glomerular infiltration by monocytes. Focal, mesangioproliferative, or membranous glomerulonephritis are less frequent and could be associated with underlying systemic autoimmune disease such as SLE [20, 91]. Laboratory testing should include evaluation of autoantibodies (ANA, anti-dsDNA, anti-ENA, aPL, and ANCA) to detect underlying systemic autoimmune disease, infectious workup for viral hepatitis (hepatitis C and B) or other potential infectious causes, and malignancy workup.

Treatment and Outcome

Treatment of cryoglobulinemic vasculitis is adapted according to the underlying mechanism and includes combination of immunosuppressive therapy to reduce immune complex-mediated inflammation, antiviral treatment for underlying HCV infection, and biologic therapy aimed at suppressing B-cell proliferation [33, 69, 83]. Milder manifestations of noninfectious cryoglobulinemic vasculitis such as purpura, livedo reticularis, and non-debilitating neuropathy are treated with low-dose corticosteroids. Cutaneous ulcers, glomerulonephritis, and progressive peripheral neuropathy are treated with corticosteroids and rituximab or cyclophosphamide [35]. Life-threatening manifestations including rapidly progressive glomerulonephritis, pulmonary hemorrhage, mesenteric vasculitis, and CNS involvement are treated with high-dose corticosteroids and plasmapheresis in combination with cyclophosphamide and rituximab [33, 69, 89]. Intravenous immunoglobulin application is contraindicated as it can exacerbate immune complex precipitation leading to multi-organ failure. Symptomatic hyperviscosity requires urgent treatment with plasma exchange [83].

The outcome strongly depends on the underlying condition and complications. Poor prognostic signs in patients with noninfectious cryoglobulinemic vasculitis include renal failure and pulmonary and gastrointestinal involvement [99]. There are no data on the long-term prognosis of pediatric patients with cryoglobulinemic vasculitis.

Conclusion

Nephropathies may occur in relation to several autoantibodies which may target renal-specific autoantigens like in anti-GBM disease or autoantigens of various tissues as in systemic autoimmune diseases. Recent developments in autoantibody detection have increased the reliability and relevance of autoantibody testing and eased the diagnosis of autoimmune nephropathies. Although seen relatively rare in the pediatric age, these diseases cause important morbidity and may lead to end-stage renal disease. Being aware of these rare entities, paying attention to kidney symptoms, and making appropriate investigations will make early diagnosis and prompt interventions possible.

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Membranoproliferative Glomerulonephritis and C3 Glomerulopathy in Children

22

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Abstract

Membranoproliferative glomerulonephritis (MPGN) and C3 glomerulopathy (C3G) define a disease pattern characterized by proteinuria, hypertension, and impaired kidney function with a significant risk of disease progression to end-stage kidney disease (ESKD) despite all current treatment efforts. While historically morphological criteria were applied to classify MPGN, recent advances in the understanding of disease pathogenesis with the appreciation of predominant glomerular C3 deposition and the identification of a key role for the complement alternative pathway (AP) resulted in disease reclassification. Today, C3G characterizes a glomerulopathy with predominant C3 deposition. It can be subdivided by electron microscopy into dense deposit disease (DDD) with electron-dense deposits located in the lamina densa of the glomerular basement membrane (GBM), and C3 glomerulonephritis (C3GN) with deposits in subendothelial, subepithelial, and/or mesangial localization. By contrast, cases with coappearance of C3 and immunoglobulins are classified as idiopathic immune-complex MPGN (IC-MPGN). Interestingly, complement mutations and C3 nephritic factor (C3NeF) – an IgG antibody binding to and stabilizing the AP C3 convertase – are found in about 20 and 80% of C3G cases, respectively, but can also be found in IC-MPGN patients. Current treatment recommendations include conservative measures in all cases, and in some immunosuppression with corticosteroids and mycophenolate mofetil (MMF). While milder in children compared to adults, overall prognosis is poor, with roughly 20% of children reaching end-stage kidney disease (ESKD) within 10–15 years. Future treatment strategies are expected to include therapies that block the AP and thereby reduce glomerular C3 accumulation.

Keywords

Membranoproliferative glomerulonephritis · Immune complex glomerulonephritis · C3 glomerulopathy · C3 glomerulonephritis · Dense deposit disease · Complement alternative pathway

Abbreviations

ACEi	Angiotensin-converting enzyme inhibitor
aHUS	Atypical hemolytic uremic syndrome
AMD	Age-related macular degeneration
AP	Alternative pathway (of complement)
aPL	Acquired partial lipodystrophy
ARB	Angiotensin II receptor antagonist
ASO titer	Antistreptolysin O titer
C	Complement
C3G	C3 glomerulopathy
C3GN	C3 glomerulonephritis
C3NeF	C3 nephritic factor
C4NeF	C4 nephritic factor
C5NeF	C5 nephritic factor
CFB	Complement factor B
CFH	Complement factor H

CFHR1-5	Complement-factor-H-related protein 1-5
CFI	Complement factor I
CFP	Properdin
CP	Classical pathway (of complement)
DDD	Dense deposit disease
DEAP-HUS	Deficiency of FHR plasma proteins and FH autoantibody positive HUS
DGKE	Diacylglycerol kinase epsilon
ESKD	End-stage kidney disease
FFP	Fresh frozen plasma
GBM	Glomerular basement membrane
GN	Glomerulonephritis
IC	Immune complex
iC3b	Inactivated C3b
Ig	Immunoglobulin
IRGN	Infection-related glomerulonephritis
LP	Lectin pathway (of complement)
MCP	Membrane-cofactor protein
MLPA	Multiplex ligation-dependent probe amplification
MMF	Mycophenolate mofetil
MPGN	Membranoproliferative glomerulonephritis
NeF	Nephritic factor
NGS	Next-generation sequencing
PMN	Polymorphonuclear leukocytes
RAAS	Renin-angiotensin-aldosterone system
SCR	Short consensus repeat
SNP	Single-nucleotide polymorphism
TMA	Thrombotic microangiopathy

Introduction

Membranoproliferative glomerulonephritis (MPGN)/C3 glomerulopathy (C3G) defines a disease pattern clinically characterized by proteinuria, hypertension, and impaired kidney function with a significant risk of disease progression to end-stage kidney disease (ESKD) in roughly 20% of children within 10–15 years from onset.

From a Morphology to a Pathogenesis-Based Disease Classification

The membranoproliferative pattern of glomerular injury has been recognized as far back as Volhard and Fahr's atlas of glomerular morphology in Bright's disease [1]. MacCallum, Bell, Ellis, Allen, and Jones described lobular and proliferative changes consistent with MPGN in the 1930s through the 1950s [2–6]. But it was not until West et al. described hypocomplementemia in children with a membranoproliferative pattern of glomerular injury that MPGN becomes a "disease" [7]. MPGN was subclassified into type I, type II, and two type III (Burkholder III and Strife/Anders III) [8–11]. Despite the insight by Johnson et al. showing hepatitis C as the cause for more than 80% patients with MPGN type I, nosology expansion continued as pathologists used the term indiscriminately as characteristic of a wide variety of disparate entities (Table 1). As expansion continued, it became clear that MPGN is not a disease, but a pattern of disease [12].

The historical classification has been replaced and is now based on pathogenesis as demonstrated by the presence or absence of immunoglobulins and complement components on renal biopsy: (1) C3 glomerulopathy (C3G) with C3 deposits alone or C3 dominant deposits; (2) immune-complex MPGN (IC-MPGN) with C3 deposits with dominant or codominant immunoglobulins/immune complex deposits; and (3) immune-negative MPGN [13, 14]. These cases are the result of endothelial injury with or without an immune-associated cause including chronic sickle cell anemia, transplant glomerulopathy, etc.

The vast majority of biopsies with an MPGN pattern will have either C3 and Ig deposits or no deposits and are secondary to any of a large number of diseases (Table 1). In rare cases, the underlying etiology of IC-MPGN cannot be determined

Table 1 Causes of secondary membranoproliferative glomerulonephritis (MPGN)

Immunoglobulin mediated
Infectious diseases
Hepatitis B [182], hepatitis C [183], hepatitis E [184], Epstein Barr virus [185], and human immunodeficiency virus [186]
Endocarditis [34], shunt nephritis [187], visceral abscess [188], and empyema [189]
Tuberculosis [190], leprosy [191]
Brucellosis [192], Q fever [193]
Malaria [194], schistosomiasis [195]
Onchocerciasis; filariasis, *Loa loa* infections [196]
Systemic immune diseases
Cryoglobulinemia [197]
Systemic lupus erythematosus [198]
IgA vasculitis [199]
Sjögren syndrome (secondary to cryoglobulinemia) [200]
Hypocomplementemic urticarial vasculitis [201]
Rheumatoid arthritis [202]
X-linked agammaglobulinemia [203]
Neoplasms/dysproteinemias
Plasma cell dyscrasia [204]
Monoclonal gammopathy of unknown significance [205, 206]
Fibrillary glomerulopathy [207]
Immunotactoid glomerulonephritis [208]
Proliferative glomerulonephritis with monoclonal immunoglobulin deposition [209, 210]
Proliferative glomerulonephritis with isolated light chain deposition [211]
MPGN with masked monotypic immunoglobulin deposits [212]
Light-chain and/or heavy-chain deposition disease [213]
Leukemias and lymphomas (with or without cryoglobulinemia) [214, 215]
Bone marrow transplant [216]
Carcinomas, Wilms tumor, and malignant melanoma [217]
Angiofollicular lymph node hyperplasia with or without TAFRO syndrome [218, 219]
Sinus histiocytosis with massive lymphadenopathy [220]
Other
Alpha-1-antitrypsin deficiency with severe liver disease [221]
Gluten-sensitive enteropathy (celiac disease) [222]
Immunoglobulin negative
Thrombotic microangiopathy including drug-induced forms [223]
Sickle-cell disease [224]
Transplant glomerulopathy [225]
POEMS syndrome [226]

TAFRO thrombocytopenia, anasarca, fever, renal dysfunction (or reticulin myelofibrosis), and organomegaly, *POEMS syndrome* polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes

and is referred to as "idiopathic IC-MPGN" [15]. However, these cases should be further examined for abnormalities of complement as they may represent C3G with some immunoglobulin deposition.

C3G is the primary focus of this chapter. It is considered a primary complement disease, where complement deposition is the result of defective control of the complement alternative pathway (AP) [16, 17].

Histopathology

C3 Glomerulopathy

Light microscopy: There are four major light microscopy patterns seen in DDD and C3GN including a mesangial proliferative pattern, a membranoproliferative pattern, a crescentic pattern, and an acute proliferative and exudative pattern (Figs. 1 and 2) [18–22]. Vargas et al. provided the first major report detailing the not uncommon finding of numerous polymorphonuclear leukocytes (PMNs) in DDD as well as the crescentic variant of DDD [23].

Immunofluorescence microscopy: Multiple studies have detailed the immunofluorescence

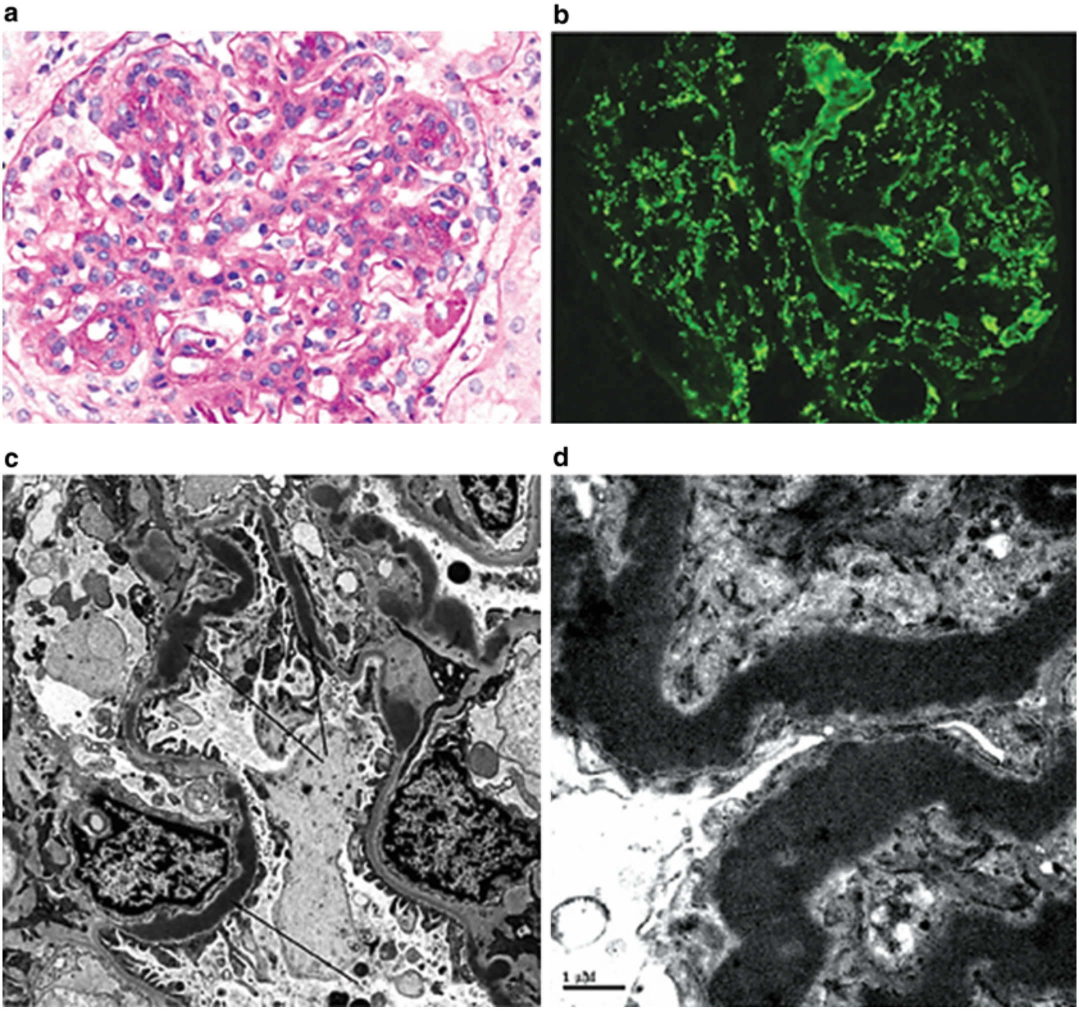

Fig. 1 Dense deposit disease: (**a**) diffuse mesangial hypercellularity with segmental endocapillary hypercellularity and pink ribbon-like enhancement of glomerular basement membranes (periodic acid-Schiff stain, original magnification 600x); (**b**) C3 granular deposits diffusely in mesangial areas and segmentally along glomerular basement membranes (fluorescein-conjugated antihuman C3, original magnification 600x); (**c**) segmental electron dense transformation of glomerular basement membranes and mesangial areas (arrows, unstained grid, and original magnification 4000x); and (**d**) diffuse electron dense transformation of glomerular basement membranes (unstained grid, original magnification 12,000x)

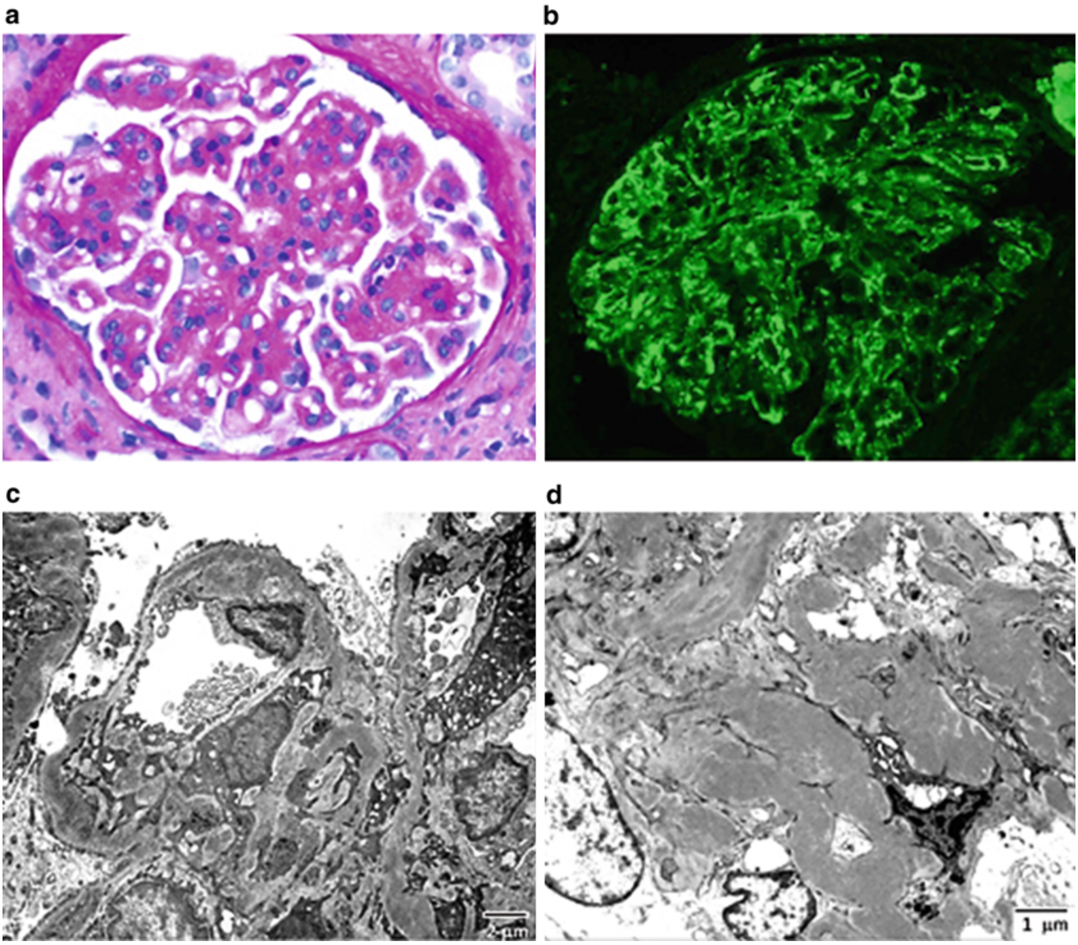

Fig. 2 C3 glomerulonephritis: (**a**) diffuse mesangial hypercellularity with endocapillary hypercellularity and thick glomerular basement membranes (periodic acid-Schiff stain, original magnification 600x); (**b**) C3 granular deposits diffusely in mesangial areas and along glomerular basement membranes (fluorescein-conjugated antihuman C3, original magnification 600x); (**c**) segmental charcoal gray transformation of glomerular basement membranes and mesangial areas (unstained grid, original magnification 4000x); and (**d**) diffuse charcoal gray transformation predominantly in mesangial areas but also within glomerular basement membranes (unstained grid, original magnification 8000x)

findings in C3G usually examining IgG, IgM, IgA, and C3c [18–22, 24–27]. C3 is intensely positive and dominant in both in the glomerular basement membranes and mesangial regions. In DDD, similar deposits may be seen along tubular basement membranes and the basement membrane of Bowman capsule. It is important to note that immunoglobulins and C1q are not uncommonly seen in C3G in a pattern similar to the C3 deposition [18–22, 24, 27].

Electron microscopy: DDD is characterized by the unique electron dense transformation of the basement membranes demonstrated using electron microscopy [10, 18–22, 28–30]. Though the diagnosis can be suspected by characteristic light and immunofluorescent features, the diagnosis must be confirmed by ultrastructural examination. The glomerular basement membranes undergo an extremely electron-dense transformation that looks as if the glomerular basement membranes had been calligraphed. In many areas, the entire length of the GBM will be replaced, while in other loops, only segments may be involved. Though the transformation usually involves the full thickness of the loop, it may include only a part. Subepithelial hump-like deposits with similar deep electron density are not uncommon [10, 18–22, 31]. In the deep mesangium, this same electron-dense transformation assumes a spheroid appearance.

In C3GN, the electron microscopic appearance of the deposits varies from an amorphous charcoal grey to well-defined and darker densities indistinguishable from immune-complex deposits [27, 32]. They are typically located in the subendothelial space and mesangium with occasional subepithelial deposits that may be hump-like.

Differentiating C3 Glomerulonephritis from Dense Deposit Disease

Dense deposit disease (DDD) is defined by the extremely electron dense transformation of the glomerular basement membranes as seen by electron microscopy, whereas C3GN is defined by the presence of C3 only or C3 dominance on immunofluorescence examination. C3GN is thus a diagnosis of exclusion since a C3-dominant pattern is significantly more common as a secondary event, particularly in infection-associated GN, rather than the extremely rare C3GN [33–35]. The use of pronase-digested paraffin immunofluorescence may reveal the presence of significant immunoglobulin deposition that would make C3GN less likely [36, 37].

Immunoglobulin-Associated Membranoproliferative GN

Immune complex-associated MPGN (IC-MPGN) is defined as a membranoproliferative light microscopy pattern with immunoglobulin dominance on immunofluorescence examination (Fig. 3). The overwhelming majority of cases are secondary (Table 1), and the presence of immunoglobulins and light chains is an important clue to the underlying condition. These should only be labeled “idiopathic” after an exhaustive clinical evaluation [15].

Membranoproliferative Glomerulonephritis Without Immunoreactants

This pattern is seen in the setting of chronic glomerular endothelial cell injury (Table 1). Residual acute changes my reveal the underlying cause. For example, segmental mesangiolysis and/or endothelial cell injury with fragmented red blood cells suggest chronic active thrombotic microangiopathy. Paraffin immunofluorescence is suggested if any electron-dense deposits are present on electron microscopy.

Transition Between IC-MPGN and C3G

Serial renal biopsies are rarely performed, and most patients are fitted into one of the above categories. However, there are multiple case reports of change over time from one form to another [38–43]. Typically, this is a change from an IC-MPGN to either C3GN or DDD (Fig. 4), though reports of infection-related glomerulonephritis (IRGN) evolving into C3G are also reported.

Lessons Learned from Animal Models

Genetically engineered and spontaneous animal models were instrumental in advancing our understanding of C3G pathogenesis. Spontaneous deposition of C3 within glomeruli has been reported in animals deficient in FH [44–47]. There was a pig strain that developed de novo FH deficiency [47] and a mouse strain (*Cfh*$^{-/-}$) generated by gene targeting in embryonic stem cells [44]. In both models, the deposition of C3 along the GBM was followed by electron-dense transformation of the GBM together with varying degrees of glomerular inflammation and structural damage (MPGN). In both models, terminal complement components were present in addition to C3, but IgG was absent in the initial lesions. Since these animals lacked FH, there was uncontrolled activation of C3 in the circulation, and consequently, levels of circulating

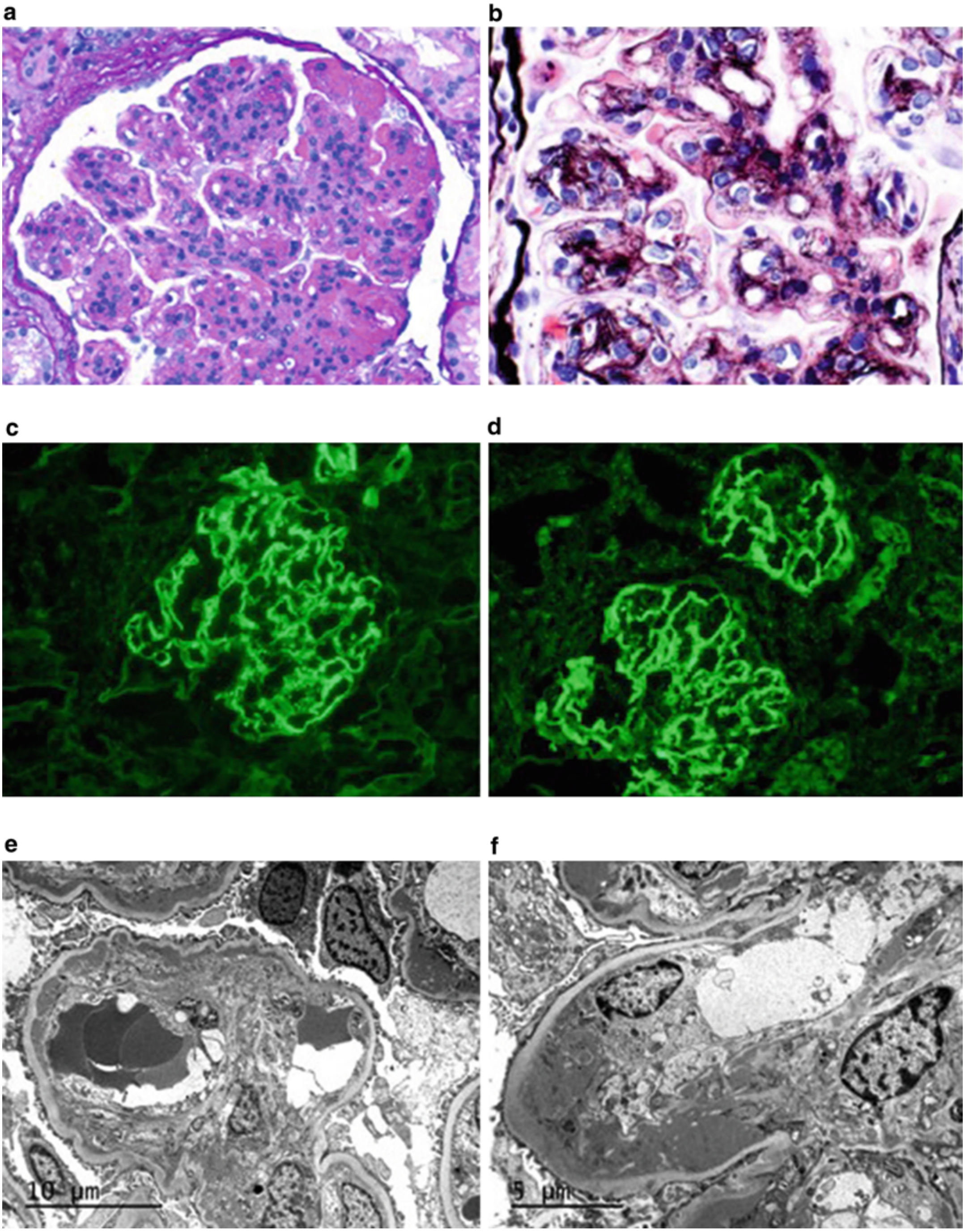

Fig. 3 Membranoproliferative glomerulonephritis, immune complex type: (**a**) mesangial and endocapillary hypercellularity with segmental lakes of pink deposits (periodic acid-Schiff stain, original magnification 600x); (**b**) glomerular basement membrane double contours with pink deposits. Mesangial and segmental endocapillary hypercellularity (Jones silver stain, original magnification 600x); (**c**) IgG granular deposits along glomerular basement membranes and in mesangial areas (fluorescein-conjugated antihuman IgG, original magnification 400x); (**d**) C3 granular deposits similar to IgG (fluorescein-conjugated antihuman C3, original magnification 400x);

C3 were extremely low. The *Cfh*$^{-/-}$ strain has been studied in detail and has provided some important insights into the pathogenesis of C3G. It is important to note here, however, that this is a model of C3G associated with complete FH deficiency. This is an extremely rare cause of C3G. So, care is needed in linking the findings from the *Cfh*$^{-/-}$ strain to human C3G.

The most obvious lesson from the *Cfh*$^{-/-}$ strain is the critical importance of FH in protecting the GBM from spontaneous C3 deposition. It is remarkable that simply removing this regulator results in florid C3 deposition along the GBM together with terminal pathway components. When C5 activation was prevented, by intercrossing the *Cfh*$^{-/-}$ strain with C5-deficient mice, the abnormal glomerular C3 remained unchanged but the severity of both spontaneous and experimental glomerulonephritis was reduced [48]. This indicated that strategies to block C5 activation might have utility in reducing glomerular inflammation in C3G but would not alter glomerular pathology driven by the abnormal C3 deposition.

In contrast to blocking C5, restoring C3 regulation in the *Cfh*$^{-/-}$ strain would be expected to ameliorate C3G. The first attempts to do this were by adding back FH. Administration of either mouse or human FH to *Cfh*$^{-/-}$ mice reduced glomerular C3 staining and increased circulating C3 levels [49–51]. Constructs that contain only the regulatory and targeting domains of FH, termed mini-FH molecules, also ameliorated glomerular C3 [52]. Other approaches were the administration of novel regulatory proteins that target to sites of complement activation. These included TT30 [53], a protein containing the complement regulatory domains of FH (FH_{1-5}) linked to the complement-binding domains of complement receptor 2; and homodimeric FH molecules [54], mini-FH molecules that contain complement-binding domains of factor H-related protein 1 (FHR1).

Another approach to prevent uncontrolled C3 activation was to block AP activation in the *Cfh*$^{-/-}$ mice by removing the activation proteins of the AP, i.e., FB or FD, or by removing properdin, the AP positive regulator. The *Cfh*$^{-/-}$ mice did not develop spontaneous C3G in the absence of FB, an essential activation protein of the AP [44]. In the absence of FD, the *Cfh*$^{-/-}$ mice did not develop C3 along the GBM but over time did develop mesangial C3 deposits and glomerular injury, suggesting that there was residual AP activity in the *Cfh*$^{-/-}$ mouse sera despite deficiency of FD [55]. Properdin deficiency in the *Cfh*$^{-/-}$ strain resulted in worsening of glomerular C3 deposition [56, 57]. This was unexpected since removal of this positive regulator of the AP C3 convertase would be expected to slow C3 activation. What are the messages from these studies for the treatment of C3G? Taken together, these data indicate that inhibiting FB or FD can modulate AP-driven glomerular C3 deposition, but inhibition of properdin could make it worse.

A further insight was derived from studying the effects of FI, the enzyme that cleaves C3b to iC3b and then to C3c and C3dg. In the *Cfh*$^{-/-}$ strain, the uncontrolled C3 activation results in C3b which is cleaved by FI to iC3b and C3dg. However, in *Cfh*$^{-/-}$ mice that are also deficient in FI, activated C3 remains as C3b only. Interestingly, while abnormal glomerular C3 developed in both strains, the C3 in the mice with combined deficiency of FH and FI was mesangial in location, while in the *Cfh*$^{-/-}$ strain the C3 was located along the GBM. These data indicated that during uncontrolled C3 activation the nature of the activated C3 fragment could influence where it accumulates in glomeruli [58].

The nature of the activated C3 is also relevant to its longevity in glomeruli. When FH was

Fig. 3 (continued) and (**e**) subendothelial and mesangial immune complex type deposit. New basement membrane formation producing double contours with cellular interposition. Endothelial cell swelling causing luminal narrowing (unstained grid, original magnification 4000x). Large subendothelial immune complex type deposit with smaller mesangial deposits (unstained grid, original magnification 8000x)

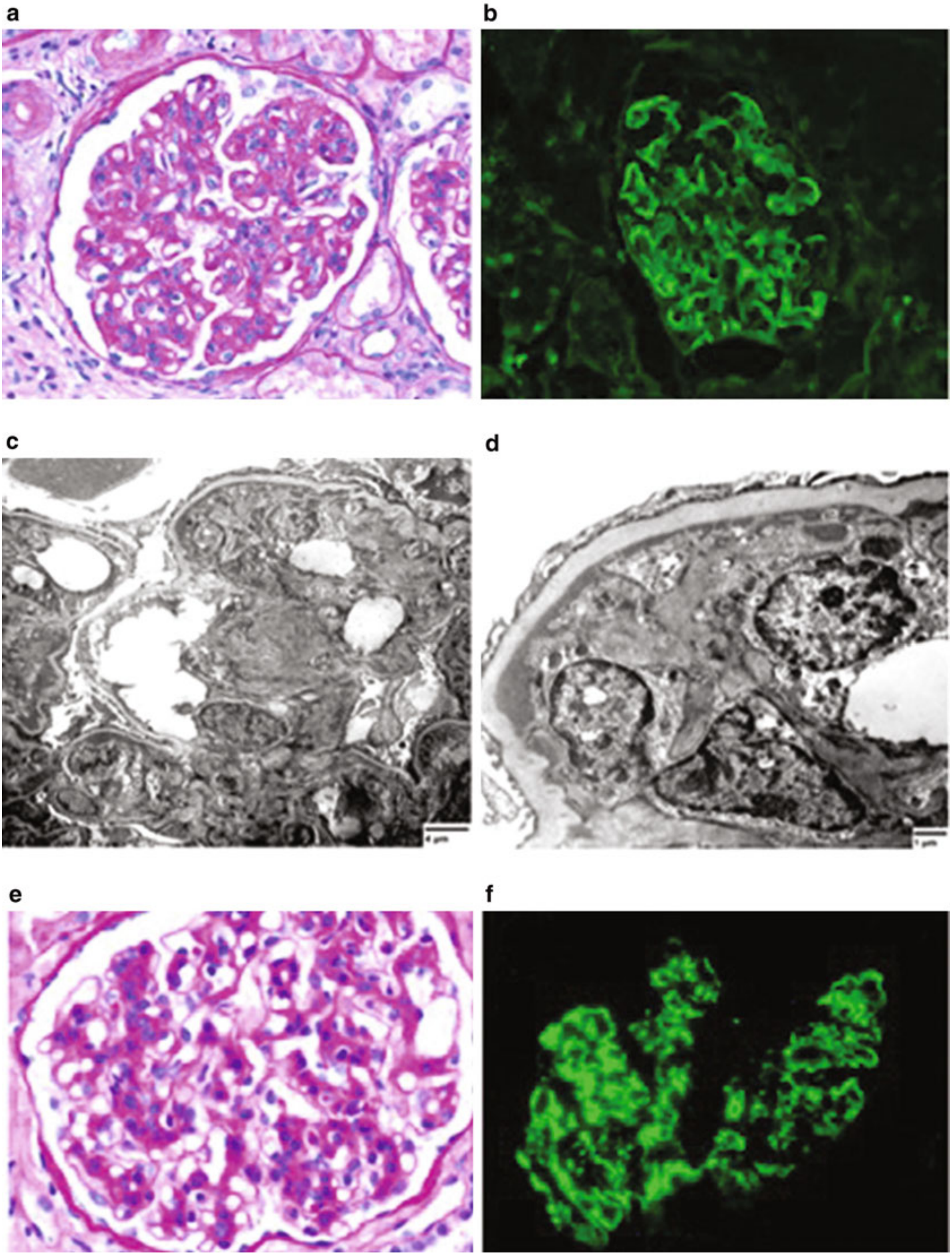

Fig. 4 (continued)

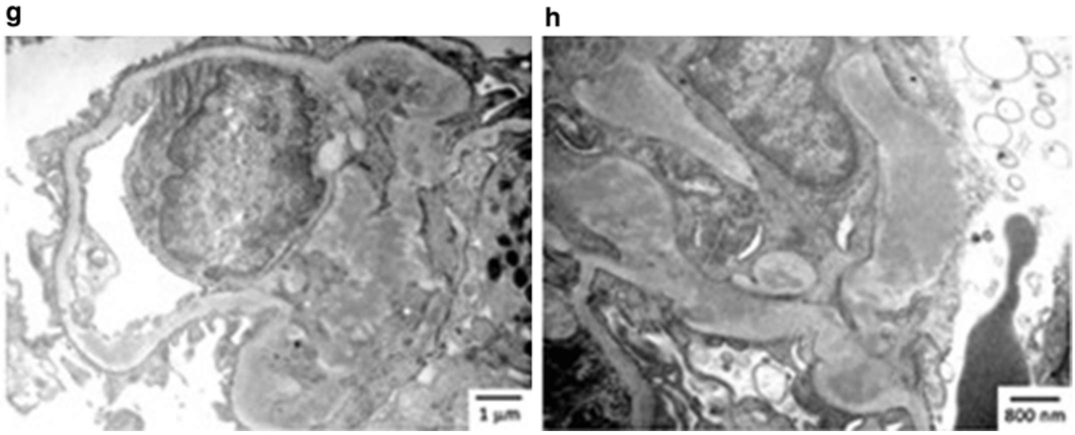

Fig. 4 First biopsy. Membranoproliferative glomerulonephritis, immune complex type (**a**–**d**): (**a**) mesangial and endocapillary hypercellularity (periodic acid-Schiff stain, original magnification 400x); (**b**) IgG deposits along glomerular basement membranes and in mesangial areas (fluorescein-conjugated antihuman IgG, original magnification 200x); (**c**) mesangial and endocapillary hypercellularity with immune complex type subendothelial and mesangial deposits (unstained grid, original magnification 4000x); (**d**) immune complex type subendothelial deposits with endocapillary hypercellularity (unstained grid, original magnification 12,000x). Second biopsy 23 weeks later. C3 glomerulonephritis (**e**–**g**); (**e**) diffuse mesangial hypercellularity with segmental, mild, endocapillary hypercellularity. No glomerular basement membrane double contours (periodic acid-Schiff stain, original magnification 600x); (**f**) C3 granular deposits diffusely in mesangial areas and segmentally along glomerular basement membranes (fluorescein-conjugated antihuman C3, original magnification 400x); (**g**) segmental charcoal gray transformation of glomerular basement membranes and mesangial areas (unstained grid, original magnification 8000x); and (**h**) diffuse charcoal gray transformation predominantly in mesangial areas but also within glomerular basement membranes (unstained grid, original magnification 12,000x)

administered to *Cfh*$^{-/-}$ mice there was a striking reduction in glomerular C3b/iC3b/C3c which was evident at 24 h. However, glomerular C3dg persisted for weeks. Similarly, in models of immune complex experimental nephritis, glomerular C3c was cleared by 85% in 24 h after cessation of complement activation, whereas glomerular C3dg persisted for weeks [59]. This is most likely because C3dg is covalently attached to surface structures within the GBM or mesangial matrix. Importantly, it tells us that the type of C3 fragment detected in the renal biopsy matters if the aim is to determine if complement activation has ceased (C3dg alone) or is ongoing (C3c and C3dg). This has clear implications for clinical trials of complement inhibitors that use renal biopsy deposition of C3 as markers of efficacy.

Mutations in FH are associated with susceptibility to atypical hemolytic uremic syndrome (aHUS) (see chapter on aHUS) indicating that defects in the complement AP can give rise to at least two distinct renal pathologies: thrombotic microangiopathy (TMA) (i.e., aHUS) and glomerulonephritis (i.e., C3G). This is explained by the observation that the majority of aHUS-associated CFH mutations do not result in complete deficiency of FH but selectively impair the surface C3b recognition domains of the protein. Mice expressing a mutant FH molecule that functionally mimicked the human aHUS-associated mutations developed TMA not C3G [48], and this was dependent on C5 [60]. These studies indicated that for aHUS to develop there need to be both defective complement regulation along the renal endothelium ("the fire") and sufficient C3 and C5 ("the fuel") to mediate the damage. In *Cfh*$^{-/-}$ mice, the absence of FH means that the FH-mediated regulation of complement along endothelium is not possible. However, these mice have very low circulating C3 and C5 levels so lack sufficient complement to cause damage and are therefore protected against aHUS. This indicates that the balance between surface regulation (preventing complement-mediated damage to surfaces like the renal endothelium) and plasma regulation (preventing depletion of C3 and C5) critically influences the nature of the glomerular pathology.

We lack models of C3G associated with C3 nephritic factors (C3NeFs). But there are two old, but important, studies of relevance. In both, the aim was to generate chronic C3 activation in an attempt to mimic the actions of C3NeF and to determine if this resulted in glomerular C3 deposition. Despite achieving prolonged periods of hypocomplementemia using either zymosan in rabbits [61] or cobra venom factor in mice [62], neither strategy resulted in glomerular C3 deposition. More recently, mice expressing human C3 have been shown to develop spontaneous C3G analogous to that seen in the *Cfh*$^{-/-}$ mice [63]. The pathology improved with anti-C5, anti-FB, and anti-C3b treatment. However, the reason for the uncontrolled C3 activation in this humanized strain was because *human* C3 cannot be regulated by *mouse* FH and *mouse* FI. So, the pathology in this model was a consequence of "de facto" FH deficiency, a consequence of interspecies differences in complement regulators. Taken together with the large data sets derived from the FH-deficient animal models, perhaps this is telling us that FH is the critical regulator of glomerular C3 homeostasis, i.e., when there is uncontrolled C3 activation but normal FH function, glomerular C3 deposition does not occur. It also raises questions about the relevance of C3NeF to pathogenesis of C3G.

We lack models of FHR-associated C3G where circulating complement is typically normal and where there appears to be local glomerular activation of complement. In these pathologies, the hypothesis is that the abnormal FHR proteins interfere with the ability of FH to regulate glomerular C3 activation [64, 65].

Lessons Learned from Patients

Complement dysregulation in C3G patients may occur via one or more of the following scenarios:

- Antibodies stabilizing the AP C3 convertase, thus prolonging the natural decay of the AP C3 convertase, rendering it overactive
- Mutations or antibodies to FH or FHR that result in the absence or loss of function of FH resulting in loss of control of the AP C3 convertase and its enhanced activity
- Mutations in C3 or FB that result in an exceedingly stable C3 convertase with prolonged decay and enhanced function

In all scenarios, the functional consequence is an enhanced activation rate of C3. Some antibodies prolong the half-life of the CP C3 convertase, or the (AP or CP) C5 convertase [66, 67]. Of note, some C3NeFs (properdin dependent) also stabilize the C5 convertase, hence called C5NeF, and result in terminal pathway activation and elevated sC5b-9 levels [68, 69].

The pathogenetic role of complement is further supported by the detection of complement alternative and terminal pathway proteins in the glomerulus of patients with C3G [16, 17].

Autoimmune Forms of C3G

In 1965, the first association of decreased serum C3 levels and MPGN was reported, followed by the hypothesis of a circulating factor in GN patients leading to increased C3 cleavage [70]. Subsequently, an antibody binding to a neoepitope of the complement AP C3 convertase (C3bBb), enhancing its stability and function (i.e., C3 activation), was detected and named C3NeF [66, 71]. Additional autoantibodies or nephritic factors were detected that prolonged the half-life of the complement AP or CP C3 or C5 convertases (Table 2). C3NeF and other autoantibodies found in MPGN were also detected in other renal diseases, such as systemic lupus

Table 2 Overview of mutations, rare variants, copy number variants (CNV), and polymorphisms associated with C3G

Gene/protein	Mutation/SNP	Function	Phenotype	References
CFH	Homo-/compound heterozygous SCRs 1–4 (regulatory domain)	Intact surface binding Reduced C3b binding Loss of FH cofactor and decay accelerating activity	C3G IC-MPGN	[68, 78, 93, 94, 96, 98, 100, 103, 104, 106]
CFI	Homozygous Heterozygous	Decreased FI-mediated C3b degradation	C3G IC-MPGN	[68, 78, 93, 94]
C3	Heterozygous	C3mut – resistant to cleavage by C3bBb C3mut convertase – resistant to FH inactivation C3 binding with FI or FH	C3GN IC-MPGN	[78, 93, 94, 105]
CFB	Heterozygous/Homozygous	Alters C3-FB interaction	C3G IC-MPGN	[78, 94]
Thrombomodulin	Homozygous	Not tested	DDD	[78, 94]
DGKE	Homozygous Heterozygous – unclear impact	Not complement mediated	MPGN	[93, 112]
At-risk SNPs (reviewed in [227]				
MCP/CD46	Rare SNP	Not tested	C3G IC-MPGN	[68]
CFH	Rare SNP, e.g., Y402H (SCR 7)	Not tested	DDD	[95, 101, 107]
CFHR5	Rare SNP	Not tested	DDD	[93, 95, 107]
C3	Rare SNP	Not tested	DDD	[95, 108]
CFHR fusion proteins (reviewed in [92]				
CFHR3-1	CNV *CFHR3-1* hybrid gene	Greater degree of FHR-mediated deregulation	C3GN	[64, 110]
CFHR2-5	*CFHR2-5* hybrid gene	Stabilizes C3 convertase, reduced FH-mediated decay	DDD	[97]
CFHR5-CFHR5	CNV Duplication in *CFHR5* exons 2/3	Greater degree of FHR-mediated deregulation	C3G	[64, 99]
CFHR1-CFHR1	Internal duplication	Greater degree of FHR-mediated deregulation	C3G	[102]
CFHR5-2	*CFHR5-2* hybrid gene	Greater degree of FHR-mediated deregulation	C3GN	[92]
CFHR1-5	*CFHR1-2* hybrid gene	Greater degree of FHR-mediated deregulation	C3G	[228]

erythematosus (SLE) [72–74], IRGN [75], meningococcal meningitis [76], and in healthy individuals, rendering the interpretation of their pathogenic value controversial [66, 77].

C3NeF is the most prevalent antibody and was detected in 86% of DDD, 46% of C3GN, and up to 50% of patients with IC-MPGN, respectively, and is associated with decreased C3 levels [68, 78]. C3NeF levels might fluctuate during the clinical course and do not necessarily reflect disease activity or treatment status [79]. As C3NeFs are heterogeneous, reliable detection is challenging, and currently no international standard for C3NeF detection exists [66]. The finding of C3NeF does not exclude the coexistence of complement mutations, and despite C3NeF positivity, comprehensive complement diagnostics should be performed (Table 4) [80].

Anti-C3b and Bb antibodies, both stabilizing the AP C3 convertase, have been reported in several patients with C3G and IC-MPGN [81, 82]. Fifty percent of those patients had a history of infection [81]. Patients with both anti-C3b and Bb antibodies presented with terminal pathway activation, and worse renal outcome was reported [81, 83]. FH autoantibodies, frequently associated with aHUS [84], were reported but are rare in MPGN and in contrast to aHUS seem to affect FH cofactor activity [85–87].

C4NeF has been mainly detected in IC-MPGN and C3G patients with a prevalence of 3–20% in different cohorts. C4NeF commonly coexists with C3NeF, and a favorable long-term outcome was reported [88–90]. C5NeF describes antibodies, stabilizing the C3 and C5 complement AP convertases, and has been detected in patients with C3G and DDD [91]. It is unclear whether this is a unique nephritic factor or represents a C3NeF with terminal pathway dysregulation [66].

Genetic Forms of C3 Glomerulopathy

Genetic studies have showed that approximately 25% of patients with C3G have rare or unique variants in complement genes [68, 92, 93]. Genetic causes are more common in C3G, than DDD, and are less likely found in IC-MPGN patients [68, 92]. In a large cohort of idiopathic/primary IC-MPGN, Iatropoulos et al. recently showed that the frequency of genetic variants in complement AP genes or C3NeF, or both, was similar to the frequency in patients with C3G [78, 94]. This study also distinguished clusters of patients based on inflammatory markers on biopsy and biochemical findings irrespective of a C3G or IC-MPGN diagnosis. Results of this study highlight shared mechanisms across the individual diagnoses within the MPGN/C3G spectrum [94]. Detailed understanding of the specific complement dysregulation seems more important than the differentiation between C3G and IC-MPGN with respect to complement-targeting therapies (Table 5).

To date, mutations have been reported in the following complement genes: *CFH*, *CFHR5*, *CFI*, *MCP*, *C3,* and *CFB* [64, 68, 95–108]. Additional mutations or internal duplications in genes encoding FH-related proteins (*CFHR*), or formation of hybrid genes, were associated with familial C3G [109, 110]. Risk haplotypes were identified in *CFH*, *C3,* and *MCP*/*CD46*, with *CFH* Y402H haplotype more frequently reported in DDD, and *MCP*-652A4G polymorphism in C3G [68, 95]. The presence of two or more complement haplotypes increased the risk of disease [68, 95].

Table 3 gives an overview of pathogenic or likely pathogenic variants, rare variants, and polymorphisms associated with C3G. Interestingly, several variants have already been described in patients with aHUS [68]. Compared to aHUS, *CFH* mutations associated with C3G are preferentially located in the N-terminal region of FH or affect Cys residues throughout the FH protein. In either case, these mutations result in the loss of function of FH

Table 3 Autoantibodies (Ab) associated with membranoproliferative glomerulonephritis (MPGN)

Antibodies	Incidence	Coexisting with C3Nef	Effect on complement	Routine testing
C3NeF [68, 77]	Common	–	Stabilizes AP C3 convertase	Yes[a]
C4NeF [88–90]	Rare	Yes	Stabilizes CP C3 and C5 convertases	No
C5NeF [66, 91]	Rare	Yes	Stabilizes AP C5 convertase	No
Anti-Factor B Ab [82, 83]	Rare	No	Stabilizes AP C3 convertase	No
Anti-C3b Ab [83]	Rare	No	Stabilizes AP C3 convertase	No
Anti-Factor H Ab [87]	Rare	Yes	Fluid phase regulation	Yes

[a] Some functional tests are only done in specialized labs

AP alternative pathway, *CP* classic pathway, *NeF* nephritic factor complement regulatory activity similar to *CFH* null genotype [68, 104, 111]. Deletions of *CFHR3-CFHR1*, unlike in (DEAP-) DEAP-HUS patients, have not been associated with FH autoantibodies in patients with C3GN [83]. Most *CFI* variants associated with MPGN were already reported in patients with aHUS, and the functional consequences in fluid phase are not clear [68].

In 2009, Gale et al. described in 26 of 84 people of Cypriotic ancestry with unexplained renal disease a variation in *CFHR5* comprising duplication in its dimerization domain [99]. Several more mutations and hybrid variants in *CFHR* genes have been detected and are associated with C3G. The role of FHR in C3G was unclear until de Jorge et al. reported that FHR1, FHR2, and FHR5 might form homo- and heterodimers among themselves and with FH [64]. In the dimer form, these proteins are able to compete with FH for C3b binding and protect C3b from inactivation and the complement AP C3 convertase from its decay. This process, termed “deregulation,” was increased in patients with *CFHR* hybrids or *CFHR5* mutations (Table 3) [64, 99, 110].

In 2013, Ozaltin et al. published several patients with MPGN with a homozygous mutation in *DGKE*, an intracellular lipid kinase that modulates phosphoinositol signaling in the plasma membrane [112]. *DGKE* mutations might also be associated with thrombotic microangiopathy [113, 114]. Bu et al. found heterozygous variants in *DGKE* in several patients with C3G, where the clinical impact is still unclear [93].

Of note, a recent study from the UK on 146 patients of European ancestry with primary MPGN did not find a significant enrichment of variants in (candidate) complement AP genes, or in genes associated with aHUS, but rather detected a significant variant in an HLA locus [115].

Clinical Features

MGPN, C3GN, and DDD are rare diseases with an individual annual incidence estimated at 1–2 per million per total population (both pediatric and adult) [116, 117]. Once thought to be primarily pediatric diseases, it is clear that they also commonly present in the adult population [68]. The recent French series noted that 39% of patients were less than 16 years at diagnosis; the mean age at diagnosis for MPGN, DDD, and C3GN was 20.7 ± 16.8 years, 18.9 ± 17.7 years, and 30.3 ± 19.3 years, respectively [68]. Estimates range from 43% to 58% of patients with DDD, and 25–54% of patients with C3GN were below 16 years at diagnosis. Within the pediatric population, DDD tends to present at a younger age, with 70% of patients diagnosed prior to 15 years [118]. There is a slight bias with males predominating in MPGN, DDD, and C3GN [68, 116]. As the large case series that discriminate MPGN, DDD, and C3GN are predominately from Northern European populations, it is not clear if there are ethnic or racial differences in disease frequency.

The clinical presentations of MPGN, DDD, and C3GN are nonspecific and show significant overlap with regard to clinical features. Depending on the case series, nephrotic syndrome has been reported as the presenting feature in 40–65% of patients with MPGN, 30–40% with DDD, and 25–40% with C3GN. Regardless of disease type, microscopic hematuria is reported in 40–75% of patients, gross hematuria in 10–20%, and non-nephrotic proteinuria in 30–40%. Presentations with acute glomerulonephritis are common in MPGN, DDD, and C3GN, with hypertension noted in 30–60%, and renal insufficiency in 20–50% patients [21, 32, 68, 116], while rare, rapidly progressive glomerulonephritis has been reported in MPGN, DDD, and C3GN.

As the classification system for MPGN has recently evolved, our understanding of the differences in the clinical presentations of MPGN versus C3GN is limited, by sparse published literature. Doubtless, there will be refinements regarding variations in presentation; however, it is unlikely that there will be unique features that clearly distinguish MPGN, DDD, and C3GN clinically. The exception to this appears to be *CFHR5*-associated C3GN which presents in childhood with persistent microscopic hematuria, synpharyngitic gross hematuria, and a strong family history of ESKD. At present, this form of G3GN has been reported primarily in the Cypriot population, but also in two patients with non-Cypriot heritage [99, 119–121].

Hypocomplementemia has usually been regarded as a distinguishing feature of MPGN I (low C3 and low C4) and other types of MPGN (low C3 and normal C4) [122]. In the current classification, MPGN is regarded as a form of immune complex disease, and depressed C4 levels are commonly held as a distinguishing feature; C3GN is thought primarily to be due to dysregulation of the complement AP, and therefore, C4 levels are usually normal. Recent retrospective case series comparing complement levels in patients with MGPN, DDD, and C3GN generally confirm the utility of C3 and C4 levels in distinguishing these disease types [68, 116]. There were, however, important caveats in these studies. In the French series (115 patients), at diagnosis, only 46% of MPGN patients had C3 levels of less than 66 mg/dl, and only 2.4% had C4 levels less than 9.3 mg/dl; low C3 levels were noted in 59% of DDD patients and 39.6% of C3GN patients. One patient with DDD and none with C3GN showed low C4 levels [68]. The series from England (80 patients) reported low C3 levels (<65 mg/dl) in 79% of DDD and 48% of C3GN patients, with suppressed C4 levels (<16 mg/dl) in 15% of DDD and 36% of C3GN patients [116]. Based on these and other reports, it appears that while assessment of C3 and C4 levels is suggestive of a diagnosis of MPGN, DDD, or C3GN, suppressed C3 or C4 levels are neither sufficient nor essential in the presentation of MPGN, DDD, or C3GN [68, 116]. It is important to recognize that of the combined patients reported in these case series with biopsy-proven MPGN, DDD, or C3GN, 43% had normal C3 levels at the time of diagnosis [68, 116]. Patients with *CFHR5*-associated C3GN are also reported to have normal serum C3 concentrations [123].

Extrarenal manifestations are uncommon in C3G. These include acquired partial lipodystrophy (aPL) and ocular C3 deposits, similar to soft drusen seen in age-related macular degeneration (AMD) [101]. Interestingly, polymorphisms in complement genes are also associated with the risk of developing AMD [124]. These drusen consist of C3 split products and FH [125, 126]. The long-term risk for visual problems is about 10% [127]. aPL usually precedes the diagnosis of MPGN and may present with low C3 levels and C3NeF. It is presumably caused by complement dysregulation on adipocytes [128, 129].

Diagnosis

The clinical presentations of MPGN, DDD, and C3GN are extremely variable and overlap with many other glomerular diseases, ranging from subtle/subclinical to acute and severe, as exemplified in the following scenarios:

- An accidental finding on routine urinalysis of non-nephrotic proteinuria, usually accompanied by microscopic hematuria.
- Macrohematuria concomitant or 2–3 weeks after an intercurrent infectious episode, most frequently of the upper respiratory tract.
- Nephrotic range proteinuria, with or without clinical signs of nephrotic syndrome and renal function impairment.
- Very rarely, nephrotic-range proteinuria in the setting of thrombotic microangiopathy.

Measuring circulating C3 and C4 is helpful; typically, these forms are characterized by low-circulating C3 with normal C4, especially in children. However, this is not always the case [68].

Diagnosis relies necessarily on a renal biopsy. For C3G, while the light microscopy picture will be that of proliferative GN of varying degrees of intensity, the diagnostic feature is intense and dominant C3 staining. As detailed above, electron microscopy allows distinction between DDD and C3GN based on the nature and location of the deposits.

IC-MPGN, characterized by intense and dominant or codominant immunoglobulin deposition, does not exclude a complement AP-driven pathogenesis [78, 94]. As described above, in some cases, a renal biopsy performed at onset can show both IgG and C3, while a subsequent renal biopsy performed in the same patient may reveal a clear C3-dominant pattern, as seen in classic C3G

(Fig. 4). When a proliferative GN due to impaired control of the complement AP is suspected based on the renal biopsy findings and clinical features, a full workup of complement diagnostics should be performed (Table 4).

Differential Diagnosis

In the pediatric population, low C3 and/or C4 levels are seen primarily in patients with either SLE, other forms of IC-MPGN, or C3G. A thorough history and physical examination with attention to extrarenal disease manifestations such as arthralgias, rash, neurologic, hepatic, lung, or cardiac involvement combined with ANA and anti-dsDNA antibody titers, laboratory evidence of hemolysis, leukopenia, coagulopathy, myositis, or hepatitis, strongly support a diagnosis of SLE as opposed to C3G. Although exceedingly rare in the pediatric population, essential cryoglobulinemia may also present with multiorgan involvement and systemic findings. Of note, IC-MPGN may present with low C3 *and* C4 levels (Table 1) [130]. Diseases presenting with low C3 and normal C4 levels are mainly IRGN, other forms of IC-MPGN and C3G. Very rarely, isolated low C3 and proliferative nephritis can be due to chronic bacteremia or shunt nephritis. The presence of persistent fevers or VA-shunts should alert the physician to the possibility of these clinical entities.

Distinguishing IRGN and C3G at disease onset can be challenging, as the clinical picture, serological finding, and renal biopsy picture are very similar. A renal biopsy is rarely required in a child with a classic course of poststreptococcal GN. If performed, LM shows an acute proliferative and exudative GN, IF most often both C3 and IgG and and EM subepithelial hump like deposits. However, in other forms of IRGN, such a pattern is actually rare and can be easily misdiagnosed as C3G, because, as discussed above, neither codominant IgG and C3 nor subepithelial humps rule out C3G. Recent evidence shows that transient anti-FB antibodies are frequently observed in children with acute IRGN, while they are very infrequent in C3G [131]. This laboratory exam, however, is available only in centers with a high degree of expertise in complement diagnostics. Ultimately, it is the clinical evolution that reveals whether the patient has an acute, self-limiting condition such as IRGN or a chronic, relapsing/remitting disease such as C3G. DDD or C3GN should be considered if the C3 level does not return to normal within 8–12 weeks of

Table 4 Diagnostic workup for IC-MPGN/C3G (modified from Vivarelli M, Thurman J; NKF Primer on Glomerular diseases)

History	Family history of hematuria, glomerulonephritis, and kidney failure
	Gross hematuria (if yes, concomitant or 2–3 weeks subsequent to infection; number of episodes)
	Infection (especially upper respiratory tract) in the preceding 2–3 weeks
	Previous urinalysis results?
Clinical examination	Signs of nephrotic syndrome (peripheral and periorbital edema, weight increase)
	Blood pressure
	Presence of frothy urine, reduction in urine output
	Extrarenal features: partial lipodystrophy, retinal drusen
Laboratory workup	Urinalysis, 24-hour proteinuria, microhematuria
	Complete blood count, urea, creatinine, protein, IgG, IgA, and IgM
	Serum C3, C4
	Antinuclear antibody, antidouble stranded DNA antibody; serology for hepatitis C and B virus; and serum protein electrophoresis
Complement workup	C3 nephritic factor (C3Nef), C4Nef, and C5Nef
	Anti-FH antibodies, anti-FB antibodies, and anti-C3b antibodies
	Soluble C5b9, FH, FI, Bb, properdin, and total hemolytic complement (CH50)
	Genetic screening (next-generation sequencing plus multiplex ligation-dependent probe amplification): *C3, CFH, CFI, CFB, MCP, CFHR1-5, THBD*

presentation or in the presence of active nephritis (relapsing macrohematuria and/or proteinuria) more than 8–12 weeks from the initial episode [75]. Elevated ASO titers, classically associated with IRGN, can be found also in C3G. A report by Medjeral-Thomas et al. noted elevated ASO titers in 43% of patients with DDD and 57% of patients with C3GN [116]. Finally, the presence of normal complement levels does not rule out C3G as it uncommonly presents in this fashion.

Therapy

No evidence-based standard treatment for patients with IC-MPGN or C3G exists, and current treatment recommendations are based on small studies and expert opinion [132]. Figure 5 proposes a management algorithm.

Supportive Therapy

All patients with IC-MPGN or C3G should receive renin-angiotensin-aldosterone system (RAAS) inhibition with angiotensin-converting-enzyme inhibitors (ACEi) or angiotensin II receptor antagonists (ARB) due to their antiproteinuric and antihypertensive effect. The use of ACEi or ARB was associated with better renal survival in C3G patients [68]. Low-salt diet and, when necessary and possible (in older children), lipid control must also be implemented.

Immunosuppressive Therapy

In children, there are multiple studies on the use of steroids in MPGN. A beneficial effect of steroids for proteinuria and long-term renal function was

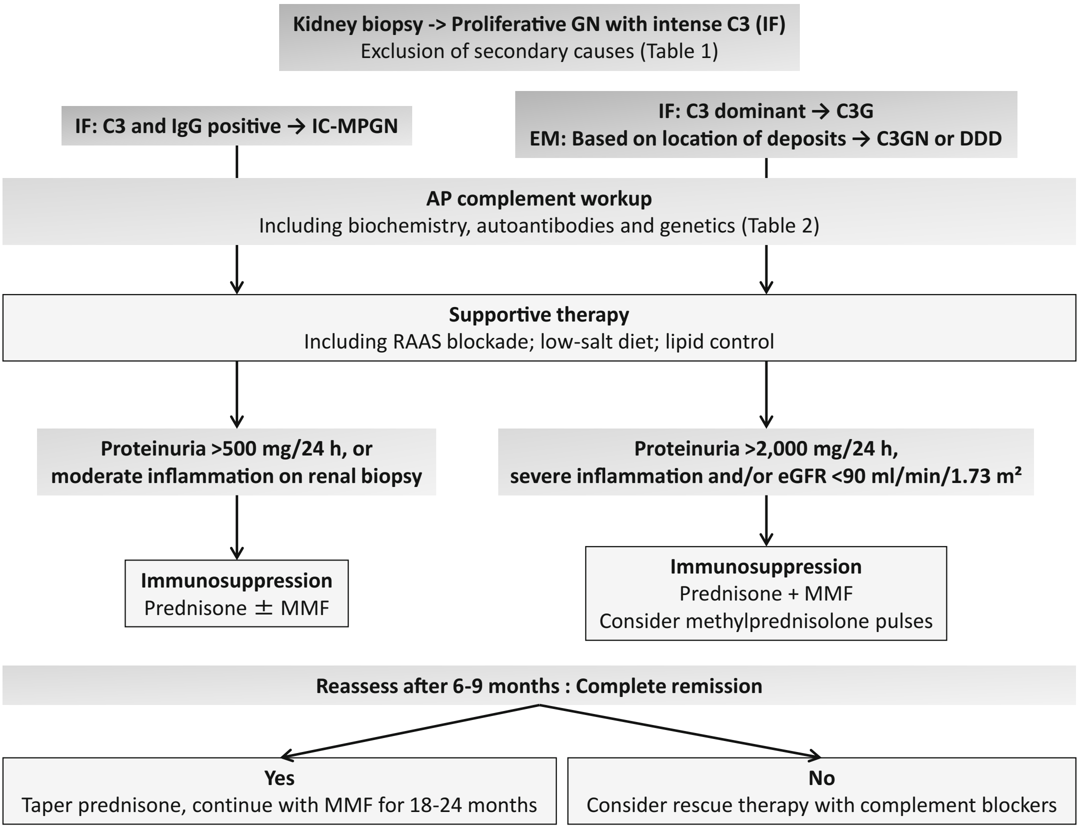
Kidney biopsy -> Proliferative GN with intense C3 (IF)
Exclusion of secondary causes (Table 1)
IF: C3 and IgG positive → IC-MPGN
IF: C3 dominant → C3G
EM: Based on location of deposits → C3GN or DDD
AP complement workup
Including biochemistry, autoantibodies and genetics (Table 2)
Supportive therapy
Including RAAS blockade; low-salt diet; lipid control
Proteinuria >500 mg/24 h, or
moderate inflammation on renal biopsy
Proteinuria >2,000 mg/24 h,
severe inflammation and/or eGFR <90 ml/min/1.73 m²
Immunosuppression
Prednisone ± MMF
Immunosuppression
Prednisone + MMF
Consider methylprednisolone pulses
Reassess after 6-9 months : Complete remission
Yes
Taper prednisone, continue with MMF for 18-24 months
No
Consider rescue therapy with complement blockers

Fig. 5 Therapeutic algorithm for IC-MPGN and C3G. For further details, please refer to paragraph on *Therapy*

detected by using long-term low-dose prednisone. However, only a subgroup of patients with MPGN type I will respond, and it may only be a partial response. Prednisone is given at a dose of 60 mg/m^2 (maximal 60 mg/d) for 4 weeks (induction), followed by 40 mg/m^2 on alternate days for another 4 weeks, and then tapered for a total of 6–12 months (maintenance). This is beneficial also in patients with C3G. It is advisable to use oral prednisone if proteinuria is >500 mg/24 h, or if the renal biopsy shows a moderate degree of inflammation. In the presence of proteinuria, >2000 mg/24 h or if the renal biopsy shows intense inflammation with endo- or extracapillary proliferation or if renal function is impaired, pulses of intravenous methylprednisolone (1000 mg/1.73 m^2 repeated 3 times), followed by oral prednisone, should be considered.

Mycophenolate mofetil (MMF) has been administered alone or in combination with prednisone in idiopathic MPGN and generated encouraging results. In 13 patients with steroid-resistant primary MPGN, the addition of MMF resulted in sustained improvement (1 year follow-up) of proteinuria and renal function [133]. In 51 patients with primary GN, including 15 patients with MPGN, partial or complete remission was reported in 70% after 1 year [134]. In a small case series of nine children with MPGN I, treatment with MMF and prednisone for a mean time of 40 months resulted in complete or partial response in five. All patients with low C3 levels were treatment failures [135]. In C3G, Caravaco-Fontan et al. recently described 97 patients (81 with C3GN, 16 with DDD), of whom 42 received corticosteroids plus MMF. This subgroup showed a higher rate of remission (79%) and a lower likelihood of renal failure (14%) compared to patients receiving other immunosuppressants, eculizumab, or conservative treatment. The superiority of this therapeutic approach was seen both in patients with genetic complement abnormalities and in those with autoantibodies to complement components [136]. The Columbia group had previously described 30 young adults with C3G treated with MMF plus corticosteroids with a median follow-up of 22 months. Twenty-two patients (67%) were defined as responders, having achieved partial or complete remission. For those who tapered MMF, relapse rate was 50% [137]. In both of these studies, heavier baseline proteinuria was a negative predictor of response.

In our experience, as is advised by the KDIGO expert opinion, MMF is beneficial in patients both with IC-MPGN and with C3G with intense proteinuria [132]. Following a prednisone taper and discontinuation over 6–9 months usually MMF monotherapy is continued for a total of 18–24 months.

Calcineurin inhibitors (cyclosporine A and tacrolimus) have been used in steroid-resistant MPGN patients. One study demonstrated its efficacy in a trial on 18 patients with refractory MPGN and low-dose prednisone therapy, by inducing long-term (2 years) reduction of proteinuria and stable renal function in 94%. Only one patient showed a recurrence after treatment discontinuation [138]. In 11 adult patients with steroid resistant, including 5 with cyclophosphamide-resistant MPGN, tacrolimus plus prednisone in 6 resulted in partial/complete remission in 9 [139, 140]. In a study on 60 patients with C3G, cyclophosphamide appeared beneficial in the subset of adolescent and adult patients [141]. In two children with MPGN and suboptimal response to prednisone, a rapid and complete remission was achieved with tacrolimus [142]. In two patients with DDD, low-dose prednisone and cyclosporine A were able to induce remission [143, 144]. On the other hand, Appel et al. did not show benefit of calcineurin inhibitors in the therapy of DDD [127]. In our experience, calcineurin inhibitors can aid in achieving remission in patients with both IC-MPGN and C3G who maintain significant proteinuria despite 6–9 months of immunosuppressive treatment.

The detection of C3NeF has prompted the use of B-cell-depleting agents like rituximab. Several case reports in patients with MPGN I, especially with IC-mediated disease, indicate partial or complete remission after administration of rituximab (in half of the cases in addition to steroids), in 11 of 13 patients [145–149]. Despite a decrease in C3NeF levels, two patients with DDD did not show any change in proteinuria or renal function [150, 151]. Of note, both patients were rescued by eculizumab therapy (below). Most authors describing treatment of C3G find no benefit in the use of rituximab, even in C3Nef positive patients. It is unclear whether this lack of response indicates that C3Nefs are not drivers of disease, or whether to be clinically effective their levels need to be brought to zero and not merely reduced.

Complement Targeting Therapy

The growing body of evidence on the role of complement AP dysregulation, in IC-MPGN and in C3G, clearly indicates that complement inhibition may be a targeted and therefore effective therapeutic strategy for these glomerular diseases. Several case reports exist on the use of plasma exchange in patients with MPGN I and DDD for disease features and recurrence in native and graft kidneys. Partial or complete remission was reported in 17 of 21 patients (81%) reported in the literature [147, 152–163]. In one patient with IC-MPGN (MPGN I) despite a transient improvement of renal function and proteinuria in response to prednisone, MMF, and plasma exchange, her condition worsened including seizures, respiratory distress, and sustained anuria. Due to deletion of *CFHR1* (without FH autoantibodies), low C3 and elevated sC5b-9, indicating complement AP and terminal pathway activation, and TMA-related symptoms, the patient was switched from plasma exchange to eculizumab. This was associated with improved clinical condition, proteinuria, and renal function [154]. One report shows the successful therapy of MPGN recurrence during pregnancy and delivery of a healthy child [164].

Three case reports, including two siblings with DDD on the background of *CFH* mutation in SCR4, and one patient with MPGN I and *MCP/CD46* mutation were treated for >3 years with chronic infusion of fresh frozen plasma (FFP). FFP as maintenance therapy, individualized per patient needs, prevented progression and maintained kidney function [100, 104, 154]. However, plasma infusions might not be enough as induction therapy [165].

Regarding complement inhibitors, the only currently available agent on the market is a terminal pathway inhibitor, the anti-C5 monoclonal antibody eculizumab and its new long-acting form, ravulizumab. In 2012, several reports of the successful use of eculizumab in patients with MPGN were published [154, 166–168]. Eculizumab is a monoclonal antibody-binding C5 and therefore preventing the assembly of the terminal complement complex (TCC, C5b-9) [169]. This complex is integrated in cell membranes and can lead to cell lysis. Its role in the pathogenesis in C3G is not known; however, elevated soluble C5b-9 levels were detected in some patients with C3G, and in animal models C5 knock-out ameliorated the disease [170].

At present, a number of small case series and single case reports have shown mixed results. Six are part of a small clinical trial while the others are single case reports [150, 154, 166–168, 171–173]. A significant response was reported in ten, a partial response in one, and no response in two of these patients, as reviewed by Vivarelli et al. [174]. These patients include cases of C3G and DDD, in native and transplanted kidney and both cases with C3NeF and genetic mutations. Taken altogether, a better outcome seemed to be associated with elevated sC5b-9 levels and shorter disease duration [174]. More recently, a retrospective French study evaluated 26 patients, 13 of whom were children, with a median treatment duration of 14 months [175]. In this study, 6 patients (23%) had a global clinical response, 6 (23%) had a partial clinical response, and 14 (54%) had no response. The patients with a global clinical response had rapidly progressive disease and more intense glomerular extracapillary proliferation, while age, extent of tubulointerstitial fibrosis, frequency of nephrotic syndrome, and frequency of alternative pathway genetic or antibody-mediated activation did not differ across the groups. These results suggest that eculizumab may target glomerular inflammation without affecting the AP complement dysregulation that is the main driver of the disease. Two patients with a mixed aHUS/C3G phenotype benefiting from treatment for eculizumab have been recently described, suggesting that eculizumab is most likely to benefit patients with an endothelial AP complement dysregulation (Busutti et al. KI Reports in print).

However, the only formal clinical use of eculizumab in IC-MPGN and C3G gave very disappointing results. The EAGLE study [176] evaluated ten patients (six with MPGN, four with C3G), all with normal renal function, intense (>3500 mg/24 h) proteinuria, and highly elevated sC5b9 (>1000 ng/ml), and receiving eculizumab for two sequential 48-week periods separated by one 12-week washout period. Primary outcome was change in 24-hour proteinuria (median of three consecutive measurements) at 24 and 48 weeks. While terminal complement pathway activation was successfully blunted in all patients, only three patients achieved partial remission of proteinuria. During the first treatment period, proteinuria, and blood levels of albumin and lipids, improved, but these benefits were lost during the washout period and never regained in seven patients.

Taken altogether, based on the information currently available, it appears reasonable to attempt eculizumab in patients with C3G/IC-MPGN only as a rescue therapy when immunosuppression has failed. Patients with intense inflammation, elevated sC5b9, recent disease onset, and/or mixed aHUS/C3G clinical presentation are more likely to respond.

A number of other complement inhibitors are being investigated in adults and adolescents with IC-MPGN and C3G. Some of these agents block the complement pathway further upstream, at the level of AP C3 convertase. In theory, these agents will be more effective than terminal pathway inhibitors, at least in a subset of patients. While results of these trials are preliminary, hopefully, more targeted therapies will be available soon (Table 5). However, nonspecific immunosuppression is effective in the majority of children with IC-MPGN or C3G, making these therapeutic options, at least for the time being, necessary only in a subgroup of patients refractory to standard therapy.

Clinical Outcomes

Data on long-term outcomes, with the new classification, is limited for C3GN and DDD. Sethi, in a series of 12 of adult patients with C3GN, reported stable short-term renal function with a mean follow-up of 26.4 months [32]. Medjeral-Thomas, comparing outcomes of DDD and C3GN in a cohort of 80 patients, reported progression to ESKD in 47% of DDD patients and 23% of C3GN patients with a median follow-up of 28 months; there was no difference in cumulative renal survival by Kaplan-Meier analysis [116]. The French cohort of 134 patients reported 10 years renal survival of 63.5%, with no difference between groups (MPGN, DDD, and C3GN) [68]. These data support that from previous reports that progression to ESKD occurs in 40–50% of patients with MPGN regardless of type within 10 years of diagnosis. Analysis by both the French and English groups suggested that eGFR and age at presentation were strongly negatively correlated with long-term renal survival [68, 116]. Medjeral-Thomas also reported that presence of glomerular crescents or dense deposits by biopsy were independent predictors of disease progression [116]. Recently, a large retrospective study comprising 165 children with both IC-MPGN and

Table 5 Therapeutic agents targeting complement activity being evaluated for C3G/IC-MPGN. (Modified from [92])

Drug	Target	Mechanism	Clinical trial number
ACH0144471	Factor D	Prevents formation of C3 and C5 convertases	NCT03369236, NCT03459443, NCT03124368
LNP023	Factor B	Prevents formation of C3 and C5 convertases	NCT03832114, NCT03955445
APL2	C3	Prevents formation of C3 and C5 convertases	NCT03453619
AMY101	C3	Prevents formation of C3 and C5 convertases	NCT03316521
OMS721	MASP2	Blocks initiation of lectin pathway	NCT02682407
Avacopan	C5aR1	Blocks anaphylatoxin formation (C3a, C4a, and/or C5a)	NCT03301467

C3G confirmed that children have a better outcome than adults [177]. After a mean follow-up of 4 years, renal function was preserved, and at 10 years, 80% of children analyzed did not meet the composite outcome of eGFR <30 ml/min/1.73 m^2, 50% eGFR reduction, or need for kidney replacement therapy [177].

Disease recurrence of MPGN and DDD in transplants is frequent; data from the ESPN/ERA-EDTA registry shows an average renal allograft loss of 32.4% at 5 years posttransplant in children [178]. Data suggesting that pediatric patients with DDD are at greater risk of graft loss from disease recurrence has also been reported in the NAPRTCS database [179]. The reason(s) for the increased risk of recurrence in children is not clear. Several limited cases series confirm an increased risk of disease recurrence for C3GN and the potential negative impact on allograft survival. Zand et al. reported outcomes on 21 patients with C3GN, and 14 (66.7%) had disease recurrence with median time to graft failure of 77 months (6.4 years) [180]. The French group reported posttransplant recurrence rates of 43%, 55%, and 60% for MPGN, DDD, and C3GN, respectively [68]. The English group reported data on 13 transplants, 6 with DDD and 7 with C3GN. All DDD patients and four (57%) with C3GN had recurrence of disease; recurrence was strongly associated with graft failure [116]. The overall allograft survival at 5 years was 69%. FHR5-associated C3GN has also been reported to recur in renal transplants [181].

Future Perspectives

MPGN is now considered a pattern of glomerular injury and no longer considered a disease. The immunohistochemical findings sort cases into different categories. IC-MPGN is most common, and the underlying cause must then be established. Immune negative MPGN is next common; usually, the underlying cause is obvious as in chronic TMA, and posttransplant glomerulopathy. This new classification also recognizes significant recent advances in the understanding of the pathogenesis of C3-mediated glomerulopathy with the identification of a central role of the complement AP. This is reflected by the finding of C3 dominance in kidney biopsy specimens (further classified as C3 glomerulonephritis [C3GN] or dense deposit disease [DDD] depending on the pattern of the electron-dense deposits). It releases C3G from a strict relationship with MPGN in that a membranoproliferative pattern may or may not be present. Significantly, a proliferative and exudative pattern or a crescentic pattern may be as common as a membranoproliferative pattern. Given this shift in our understanding of the disease processes, we envision the elimination of the term MPGN. It will remain in descriptions and perhaps even in comments but will no longer be considered a disease.

C3 activation and generation of its split products has identified a specific and novel treatment strategy for this disease spectrum – blockade of C3 activation at the level of the AP C3 convertase – a potential breakthrough in disease management holding promise for improved patient outcomes in a group of diseases characterized by its progressively deteriorating nature.

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Thrombotic Thrombocytopenic Purpura, Atypical Hemolytic Uremic Syndrome, and Spectrum of Thrombotic Microangiopathy

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Abstract

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are thrombotic microangiopathies (TMAs) which are characterized by microangiopathic hemolytic anemia (MAHA) and thrombocytopenia and show significant overlap in clinical presentation but are distinct in their underlying pathology.

TTP is caused by an inherited or acquired deficiency in *a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13* (ADAMTS13). ADAMTS13 cleaves von Willebrand factor (VWF) when released from endothelial cells, thus preventing the accumulation of prothrombotic ultra-large VWF (ULFWF) multimers. By difference, hemolytic uremic syndrome (HUS) is caused by infections with Shiga toxin (Stx)-producing *Escherichia coli* (*E. coli*; STEC HUS). And in the atypical form of HUS (aHUS), the endothelial injury is caused by complement dysregulation due to genetic (gain- or loss-of-function mutations) or autoimmune (inhibiting autoantibodies) causes. Recently, transient complement dysregulation has also been recognized in the absence of mutations and autoantibodies but rather as a consequence of infections, autoimmune diseases, drugs, mechanical endothelial injury, etc., thus defining a spectrum of secondary TMAs.

The advent of high precision medications which allow for the safe and efficient treatment of aHUS and TTP has fundamentally changed patient outcomes and quality of life.

Keywords

Thrombotic thrombocytopenic purpura · Thrombotic microangiopathy · Hemolytic uremic syndrome · Shiga toxin · Atypical hemolytic uremic syndrome · Complement

Introduction

Thrombotic microangiopathies (TMAs) are a heterogeneous group of diseases that are characterized by microangiopathic hemolytic anemia (MAHA) and thrombocytopenia. TMAs occur ubiquitously but – for unknown reasons – most frequently affect the kidneys. While spanning a wide spectrum of underlying etiologies, pathomechanisms, and clinical presentations, all TMAs have the formation of microthrombi due to endothelial cell injury as a central pathologic feature in common.

Hemolytic uremic syndrome (HUS) is caused by infections with Shiga toxin (Stx)-producing *Escherichia coli* (*E. coli*; STEC HUS). In the atypical form of HUS (aHUS), the endothelial injury is caused by complement dysregulation due to genetic (gain- or loss-of-function mutations) or autoimmune (inhibiting autoantibodies) causes. Recently, transient complement dysregulation has also been recognized in the absence of mutations and autoantibodies but rather as a consequence of infections, autoimmune diseases, drugs, mechanical endothelial injury, etc., thus defining a spectrum of secondary TMAs.

By difference, thrombotic thrombocytopenic purpura (TTP) is caused by an inherited or acquired deficiency in *a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13* (ADAMTS13). ADAMTS13 cleaves von Willebrand factor (VWF) when released from endothelial cells, thus preventing the accumulation of prothrombotic ultra-large VWF (ULFWF) multimers.

The advent of high precision medications which allow for the safe and efficient treatment of aHUS and TTP has fundamentally changed patient outcomes and quality of life. However, prohibitively high price levels seclude countries with limited resources from access to these medications – a scandalon, which to overcome remains an urgent task for the global medical community.

This chapter will review the different etiologies, clinical manifestation, management, and therapy of TTP, aHUS, and the so-called TMA spectrum of diseases. STEC-associated and other infection-associated HUS are reviewed in a separate chapter of this textbook.

Thrombotic Thrombocytopenic Purpura

Introduction

Thrombotic thrombocytopenic purpura (TTP) was first described in 1924 by Dr. Eli Moschcowitz [1]. He reported on a 16-year-old female presenting with unexplained fever, pallor, and petechiae. She developed unilateral weakness, followed by coma, and died a day after coma onset. On autopsy, diffuse arteriolar and capillary thrombotic occlusions were found, including the heart and kidneys; the brain was not examined. Initially termed Moschcowitz syndrome, the term TTP was proposed in 1947 [2].

It was initially assumed that TTP was an acquired condition (correct in the majority of cases); however, congenital disease was suspected in the 1960s with further research establishing this in the subsequent decades [3, 4], and given the eponym, Upshaw-Schulman syndrome (USS) in recognition. In these chronic relapsing patients, it was identified that plasma infusion would alleviate the thrombocytopenia and clinical features [3–6]. In 1982, Moake et al. reported their discovery of ultra-large VWF (ULVWF) multimers in patients with presumed hereditary TTP [7]. In 1996, two groups identified the VWF-cleaving protease [8, 9] and further analysis revealed this to be ADAMTS13 (*a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13*) [10–13].

Pathophysiology

Hepatic stellate cells are the main source of ADAMTS13, with small amounts also produced in the endothelium [14]. It is released as an active enzyme in closed formation. To properly cleave VWF, its only substrate, multimers need to be unraveled by shear stress to expose A2 domains [15]. TTP is caused by a severe deficiency of ADAMTS13 either due to biallelic germline mutations in *ADAMTS13* or, more commonly, secondary to circulating autoantibodies. Deficient ADAMTS13 activity permits ULVWF multimers to circulate without proper cleavage, thereby causing diffuse platelet aggregation and microthrombosis [15, 16].

Congenital TTP (cTTP) is caused by homozygous or compound heterozygous mutations in the *ADAMTS13* gene on chromosome 9q34. Congenital disease comprises about a third of cases in children and 5% in adults [17]. Over 200 *ADAMTS13* mutations have been identified, without concentration in any specific domain for those diagnosed in childhood [18]. There are some mutations with greater frequency based on ancestry. The most common mutations in those of European descent are c.4143_4144dupA and p.R1060W, the latter of which is most prevalent (up to 1%) [19, 20]. Patients with cTTP and homozygous for p.R1060W have greater residual ADAMTS13 activity (5–10%); this is one of the most common mutations found in those presenting in adulthood, usually during pregnancy

[21, 22]. Immune-mediated TTP (iTTP) is caused primarily by neutralizing IgG anti-ADAMTS13 autoantibodies [23]; however, some antibodies are non-neutralizing and instead accelerate ADAMTS13 clearance [24, 25]. As opposed to cTTP, in which mutations are broadly distributed across the gene, anti-ADAMTS13 antibodies causing iTTP are primarily directed against the protease spacer domain [24, 26].

Diagnosis

In 1966, Amorosi and Ultmann proposed a "pentad" associated with TTP: thrombocytopenia, microangiopathic hemolytic anemia, neurologic symptoms, renal failure, and fever [27]. Though these features are variably present in TTP, they are not specific. Some are present in fewer than half of TTP cases, and a full pentad is uncommon, found in only <5% of cases [28]. Scores have been proposed, which predict severe ADAMTS13 deficiency and can help distinguish between TTP and other TMAs. The "French score" identified severe thrombocytopenia ($<30 \times 10^9$/L), serum creatinine <200 μmol/L, and positive anti-nuclear antibodies (ANA) as predictive. The presence of one or more criteria had an 85% positive predictive value, 93.3% negative predictive value, 98.8% sensitivity, and 48.1% specificity. With all three criteria met, the positive predictive value for severe ADAMTS13 was 98.7% [29]. In 2017, the seven-point PLASMIC score was proposed. Similar to the French score, platelets <30 x 10^9/L and non-severe renal involvement (creatinine <176.84 μmol/L) were found predictive; other criteria included MCV <90, INR <1.5, evidence of hemolysis (undetectable haptoglobin, reticulocytes >2.5%, and/or indirect bilirubin >2.0 mg/dL) and no history of bone marrow or solid organ transplantation [30]. A PLASMIC score of 6–7 denotes a high risk of severely deficient ADAMTS13 activity. The score was useful in pediatric patients as young as 3 years old [31], though a modification was proposed replacing MCV with the absence of bloody diarrhea [32].

TTP is confirmed by assessing ADAMTS13 activity, with severe deficiency (<10%) diagnostic in the appropriate clinical context. Most assays are ELISA and FRETS-based [33]. The presence of anti-ADAMTS13 antibodies can be assessed with ELISA or Bethesda assays, though the latter would miss non-neutralizing antibodies [25]. Antibodies may also be undetectable in iTTP if they are non-IgG, bound to ADAMTS13 forming immune complexes, or if the assay used is not sensitive. Persistent ADAMTS13 deficiency in the absence of autoantibodies suggests cTTP; for confirmation, *ADAMTS13* genetic analysis is required [34].

Management

Acute cases of immune-mediated or undifferentiated TTP should receive urgent therapeutic plasma exchange (TPE), which drastically improves survival [35]. One should not await results of the ADAMTS13 activity, which should be drawn prior to starting TPE. If there is to be any delay in transfer to an apheresis center, plasma infusion should be used to temporize. TPE should be provided daily at 1–1.5 plasma volumes with frozen plasma as a replacement until clinical response is achieved (sustained platelet count $>150 \times 10^9$/L, LDH $<1.5 \times$ ULN, and stabilization or improvement of end-organ involvement) [36]. Glucocorticoids are recommended if iTTP is suspected to decrease anti-ADAMTS13 antibody production [37]. Higher doses (e.g., 10 mg/kg) may have greater efficacy than the standard 1 mg/kg prednisone [38]; however, the use of high-dose pulsed methylprednisolone is often reserved for more severe presentations and must be balanced with possible greater toxicity.

Beyond prednisone, the anti-CD20 antibody, rituximab, is often used for immunosuppressive therapy. In many jurisdictions, it is available during the initial presentation, whereas in others approval is only granted for refractory or relapsed iTTP. Use is associated with shorter hospital stays, reduced TPE requirements, fewer relapses, greater time to relapse [39–42], and perhaps reduced mortality [43]. Other interventions can be considered for relapsed and refractory iTTP, including pharmacologic (e.g., cyclosporine, cyclophosphamide, bortezomib) and surgical options (e.g., laparoscopic splenectomy), though their benefit over rituximab is unclear [44]. Following the response to therapy, patients are monitored both clinically and by serial ADAMTS13 measurements, which should parallel clinical remission (Fig. 1). In those iTTP patients without ADAMTS13 normalization, vigilant monitoring is required to watch for relapse, particularly with exposure or reexposure to known triggers.

In cases of known cTTP, patients can be treated with plasma infusion instead of TPE. The volume of infused plasma per treatment is commonly 10–15 mL/kg, though some will use more (20–40 mL/kg) [45]; the patient's ability to handle such volumes must be assessed carefully. As with TPE, infusions should be continued until there is a clinical response. Beyond acute presentations, some patients with cTTP may require continued prophylactic/preventive infusions, typically every 2–3 weeks, based on platelet count and symptoms [19, 20]. More frequent infusions are often required during pregnancy, particularly following the first trimester [22, 46, 47]. The half-life of ADAMTS13 is 2.5–5.4 days [48–50]. Therefore, baseline activity will return approximately 5–10 days after each 10–15 mL/kg infusion [34, 50]. More recently, the use of recombinant ADAMTS13 has been shown effective and safe [51], with larger clinical trials ongoing at the time of writing.

A more recent addition to the TTP treatment arsenal is caplacizumab, a nanobody that targets

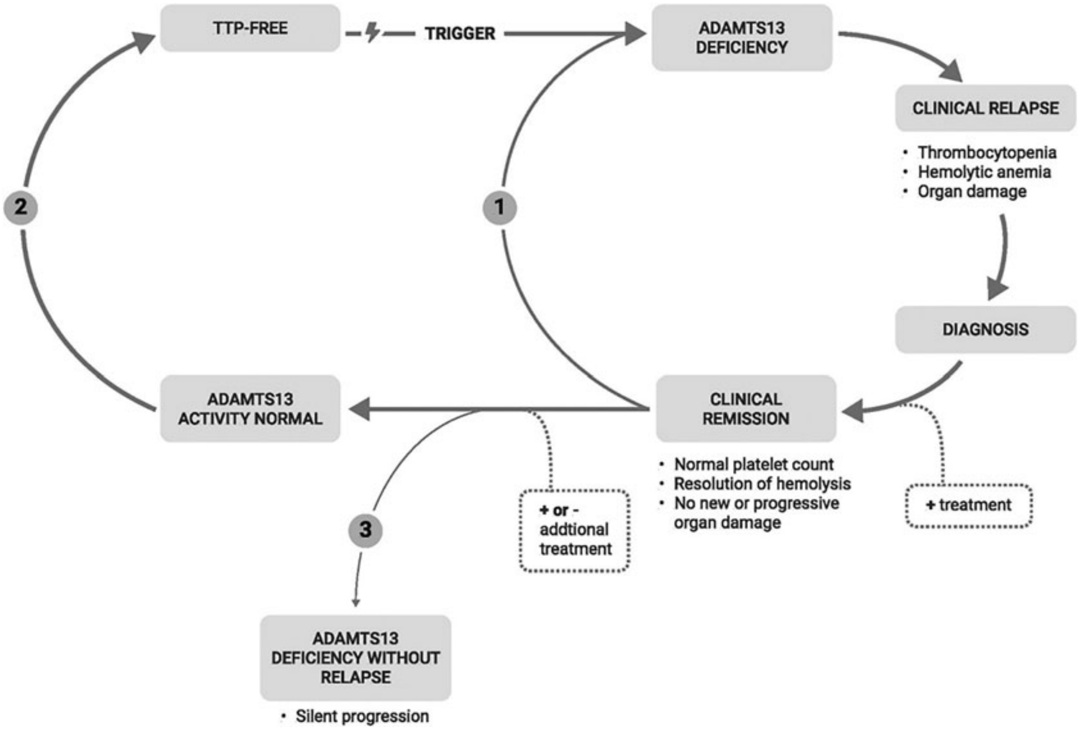
TTP-FREE
TRIGGER
ADAMTS13 DEFICIENCY
CLINICAL RELAPSE
• Thrombocytopenia
• Hemolytic anemia
• Organ damage
DIAGNOSIS
CLINICAL REMISSION
• Normal platelet count
• Resolution of hemolysis
• No new or progressive organ damage
+ treatment
+ or - addtional treatment
ADAMTS13 ACTIVITY NORMAL
ADAMTS13 DEFICIENCY WITHOUT RELAPSE
• Silent progression
1
2
3

Fig. 1 Timeline and outcomes of acute episodes of iTTP. With or without a precipitating trigger, a patient can develop acute iTTP. Following diagnosis and appropriate treatment, most patients will reach clinical response, defined as normalization of platelet count and hemolytic markers with no evidence of new organ injury. After reaching clinical response, the majority of patients (about 80%) attain normal ADAMTS13 levels (ADAMTS13 remission) and enter remission. Approximately 20% will have a persistent deficiency and may experience an exacerbation. With intensified immunosuppression and time, most will achieve clinical and ADAMTS13 remission. A small minority (<5%) will have persistent ADAMTS13 deficiency and remain at a high risk of relapse, and may also experience silent disease progression. Not depicted: Approximately 40% of the patients who achieve clinical remission will experience at least one relapse in their lifetime. (Figure reprinted from Ref. [44])

the VWF A1 domain [52, 53]. Compared to placebo, its addition to the standard of care significantly shortened platelet normalization time and reduced exacerbations. Almost all recurrences seen in the phase 2 TITAN trial occurred in patients whose ADAMTS13 activity was still <10% [52]. The phase 3 HERCULES trial permitted the extension of daily subcutaneous caplacizumab beyond the initial 30 days if the patient's ADAMTS13 had not yet recovered [53]. Caplacizumab-treated patients in the HERCULES trial had a lower rate of a composite of TTP recurrence, death, and thromboembolic disease (12% vs. 49%), driven primarily by lower recurrence. The main side effect, seen in approximately half to two-thirds of patients, was mild-to-moderate mucocutaneous bleeding [52–54]. Successful and safe use of caplacizumab has also been reported in several cases of pediatric TTP [55–59]; however, no clinical trial data exist to date.

Complement and the Link to Disease

In the beginning of the twentieth century, the complement system was first identified as the heat-sensitive fraction of human plasma that "complemented" antibodies in their ability to kill microbes. More than 50 proteins that make up the three converging complement cascades were identified and characterized; their functions were limited to surveillance of microbial intruders and the propagation of immune and inflammatory responses. The antimicrobial properties of the complement system proteins were preserved over 500 million years of evolution [60]. The past decade has improved our understanding of the evolution of the complement system, and its cross-talk with other immune and thrombotic pathways (immuno-thrombotic continuum) [61, 62], homeostatic and intracellular activities [63–66], and association with a variety of diseases [67–71].

At its most basic level, the complement system is made up of three converging pathways of fluid-phase and membrane-bound proteins whose cascading activities progress in three stages: initiation, amplification, and termination. Initiation occurs when either pathogen- or damage-associated molecular patterns (PAMPs or DAMPs, respectively) are detected by surveillance proteins. The classical pathway is initiated when the C1 complex, made up of a C1q hexamer and two of each proenzyme, C1r and C1s, binds with an antigen-antibody complex. The conformational change that occurs upon binding causes C1r to autocatalytically cleave and in turn cleave the C1s proteins. The cleaved C1s proteins then perpetuate the activation by cleaving the next set of proteins, C2 and C4, into C2a + C2b and C4a + C4b. The final step of classical pathway initiation is the coming together of C2a and C4b. This complex binds to the cell surface of the antigen-antibody complex via C4b, and C2a provides the serine protease activity that will cleave C3 as a C3 convertase (C2aC4b) moves the classical pathway into the amplification phase.

The lectin pathway is initiated by contact with microbial carbohydrates via two protein families: ficolins (ficolin-1, ficolin-2, and ficolin-3) and collectins (mannose-binding lectin, collectin-10, and collectin-11). The structure of these proteins is a mirror image to that of C1q, and they interact with analogous proteins to C1r and C1s, MASP-1 and MASP-2, which also result in the cleavage of C2 and C4. The end result of the initiation of the lectin pathway is also a membrane-bound C3 convertase made up of C2aC4b.

The alternative pathway lacks a specific initiation phase and is constitutively active. However, it provides the substrates for amplification for all three pathways as they converge on the production of C3 convertases. The alternative pathway is continuously "ticking over" via spontaneous hydrolysis of C3 (Fig. 2), which yields the cleavage product C3a (an anaphylatoxin) and C3b (the major component of the downstream cascade). Normally, C3b binds indiscriminately to both pathogens and host cells. In combination with factor D (FD), C3b facilitates the cleavage of factor B (FB) to produce fragment Bb. Bb can then form a complex with C3b to generate yet another C3 convertase, namely C3bBb.

Activation of C3bBb results in a dramatic increase in the cleavage of C3 into C3a and C3b. Either prompted via the C3 convertase of the

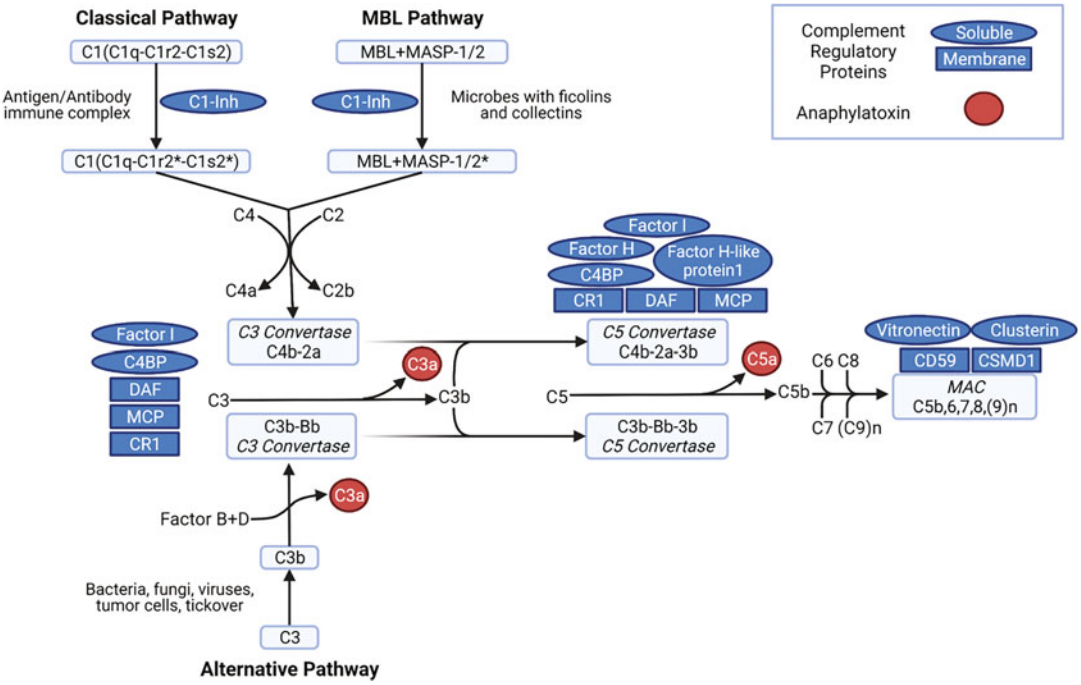
Classical Pathway
C1(C1q-C1r2-C1s2)
Antigen/Antibody immune complex
C1-Inh
C1(C1q-C1r2*-C1s2*)
MBL Pathway
MBL+MASP-1/2
C1-Inh
Microbes with ficolins and collectins
MBL+MASP-1/2*
Complement Regulatory Proteins
Soluble
Membrane
Anaphylatoxin
C4
C2
C4a
C2b
Factor I
Factor H
Factor H-like protein1
C4BP
CR1
DAF
MCP
C3 Convertase C4b-2a
C5 Convertase C4b-2a-3b
Factor I
C4BP
DAF
MCP
CR1
C3a
C3
C3b
C5a
C5
C5b
C6 C8
C7 (C9)n
Vitronectin
Clusterin
CD59
CSMD1
MAC C5b,6,7,8,(9)n
C3b-Bb C3 Convertase
C3b-Bb-3b C5 Convertase
C3a
Factor B+D
C3b
Bacteria, fungi, viruses, tumor cells, tickover
C3
Alternative Pathway

Fig. 2 **The alternative pathway of complement**. The alternate pathway of complement (AP) begins with spontaneous hydrolysis of C3 and the production of C3b. C3b combines with Bb to form the C3 convertase. The C3 convertase may go on to form the C5 convertase (C3bBbC3b) after the addition of another C3b or it may feedback to make additional C3b (the so-called amplification loop). C5 convertase will cleave C5 producing C5b, the first protein in the terminal complement complex. The ultimate products of the AP include two important anaphylatoxins (C3a and C5a) and the C5b-C9 complex. Factor H is the major regulator of the AP, and dysregulation of FH is the most common genetic cause of aHUS. (Figure created with BioRender.com)

classical and lectin pathways (C2aC4b) or the C3 convertase of the alternative pathway (C3bBb), it is this step in the alternative pathway that is designated the amplification loop. The cleavage of C3 will beget more Bb and more C3 convertases.

A small portion of the C3 convertases moves forward in the pathway to the termination stage. They do so by forming the C5 convertase (C3bBbC3b) via the addition of an additional C3b to the existing C3Bb complex. The C5 convertase in turn is responsible for cleaving C5 into C5a (another potent anaphylatoxin) and C5b (the first protein in the terminal complement complex). C5b binds to C6, C7, C8, and C9b to form the membrane-attack complex (MAC). Activation of the alternate pathway and formation of MAC causes irreparable cellular damage and is an efficient line of defense against a variety of pathogens.

In contrast to the coagulation cascade, there are no products in the complement cascade that provide negative feedback. Complement activation and amplification are only controlled by the presence of a number of regulatory proteins. The classical and lectin pathways are regulated by the C1 inhibitor binding to C1r/C1s or the MASP proteins, respectively. There are many regulators of the amplification loop, including an array of membrane-bound or soluble proteins that irreversibly dissociate the C3 and C5 convertases (decay-accelerating factor, complement receptor 1, factor H and factor H-like protein 1, C4 binding protein) or act as cofactors for factor I-mediated degradation of C3b and C4b (membrane cofactor protein, factor H, complement receptor 1, C4 binding protein) [72, 73]. Clusterin, vitronectin, CD59 and CUB, and Sushi multiple domains 1 (CSMD1) disrupt the termination stage by preventing MAC assembly [74]. Human disease is observed when there is an imbalance of positive or negative regulators of the complement system [69].

These pathways have been considered to proceed autonomously in the systemic circulation with proteins produced by the liver. It is now well established that their functions are simultaneously more expansive and local. For example, in addition to initiation of the classical cascade, C1q has many immunoregulatory functions, including enhancing phagocytosis of apoptotic and cancerous cells, regulating cytokine production by antigen-presenting cells, and altering T-lymphocyte maturation [75]. C1q also plays a critical role in many physiologic and pathologic processes, ranging from fetal development and pregnancy to tissue repair and central nervous system homeostasis [75]. MASP-1 of the lectin pathway has been described as "promiscuous" interacting with numerous substrates and inhibitors, including fibrinogen, factor XIII, and prothrombin and antithrombin from the coagulation cascade [76]. MASP-1 also can increase inflammation via binding to proteinase-activated receptors on endothelial cells and enhancing bradykinin release. Similarly, proteins of the coagulation cascade directly affect the complement cascade. Thrombin, plasmin, kallikrein, and factors XI, X, and IX might cleave C3 and C5 directly, albeit less efficiently compared to the convertases [77]. Thus, the cascades are intertwined with other immunothrombotic surveillance systems. Contrarily, at the intracellular and local levels, it has been established that most nucleated cells produce a variety of complement proteins called the *complosome*, which performs cell-modulatory functions [78]. Thus, traditional cascades are just one piece of the overall effect each complement protein has on both health and disease.

It is within this complex and evolving context that we then consider the means by which complement contributes to disease. These mechanisms have been aptly simplified into four categories: excessive activation (hyper-acute inflammatory process, such as sepsis), misdirected activation (response to a biomaterial, such as a renal transplant or dialysis filter membrane), insufficient regulation (genetic mutation in or antibody against a regulatory protein) or, in rare cases, unwarranted regulation (immunologic evasion of tumors and pathogens) [69]. Given the pervasiveness of these proteins, diseases can be local or systemic, and depending on the trigger, can be acute or chronic.

Specific to pediatric nephrology, complement dysregulation typically manifests as thrombotic microangiopathy, which is characterized by thrombocytopenia, microangiopathic hemolytic anemia, and organ injury, including acute kidney injury. Clinical findings fell into three categories: illness with a severely low ADAMTS13 and neurologic changes (thrombotic thrombocytopenic purpura, TTP), HUS in presence of Shiga toxin-producing *Escherichia coli* (STEC-HUS), and broadly all other causes of TMA (atypical HUS). This nomenclature has evolved in parallel with our understanding of the complement system and the molecular bases of TMA. Multiple similar schemas have been introduced [68, 79–82]. Another classification includes: (i) complement-mediated primary TMA, either inherited or acquired via autoantibodies, (ii) secondary TMA due to coexisting conditions, and (iii) infection-associated TMA.

Atypical HUS

Epidemiology

Most experts agree that aHUS accounts for ~5% of all patients presenting with the clinical findings of HUS. Being an ultra-rare disease, it is challenging to calculate the exact incidence of aHUS. It is presumed to be approximately two cases per one million population by extrapolating from the number of cases of non-TTP and non-Shiga toxin HUS, most of which are presumed to be due to aHUS [83]. Incidence based on European data is as high as seven per million children [84]. These are likely underestimations of the true incidence since we now know that aHUS patients can present with prodromal diarrhea [85]. On that basis, terminologies diarrhea-positive (D+) and diarrhea-negative (D-) were phased out in the past ~10 years [86]. Formerly considered as a disease of children, it is now clear that aHUS affects both children and adults approximately equally [85–89]. Males and females are equally represented in all studies. A few factors increase the likelihood that a patient with a new clinical diagnosis of HUS will ultimately be confirmed to have aHUS: presentation before ~12 months of age [90, 91], positive family history of HUS (in up to 30% of aHUS patients) [88], and history of consanguinity.

Clinical Features

Patients with aHUS often present suddenly, with many constitutional symptoms including fatigue and anorexia. Families often report a preceding illness, particularly a respiratory illness or a gastrointestinal infection (causing non-bloody diarrhea) [85, 89, 93–98]. The tight relationship of aHUS episodes with various triggering infections may initially suggest other forms of HUS (e.g., STEC or pneumococcal HUS), leading to diagnostic confusion and delays to implement effective therapies.

The classic diagnostic triad of TMA is usually evident at the initial presentation. Pallor due to the microangiopathic hemolytic anemia is usually noted. A bleeding diathesis may be documented, linked to ongoing thrombocytopenia. Manifestations of acute kidney injury (AKI) are common, including hypertension, decreased urine output, and edema [88, 89]. It is important to acknowledge that in 10–20% of patients, bona fide aHUS can present without thrombocytopenia: “partial aHUS” invariably has evidence of microangiopathic hemolysis and AKI, and clinical outcomes are similar to that of patients with “full aHUS” [99].

While the kidneys are the primary target in all forms of aHUS, extrarenal involvement is present in 20–50% of patients [100]. The range of extrarenal organs affected in patients with aHUS closely overlaps with that reported for patients with STEC HUS [101]. The most frequent extrarenal symptom is seizures, but other neurologic abnormalities may also be observed (diplopia, blindness, paresis, coma). Prompt neuroimaging is invaluable to discern if these events are due to CNS thrombosis or a result of underlying kidney pathology (e.g., posterior reversible encephalopathy syndrome, PRES) [102]. Cardiac dysfunction, including pericardial effusion or myocardial infarction, has been reported [103]. Gastrointestinal involvement may occur in the form of hepatitis, pancreas necrosis, and/or ischemic colitis [100]. Vasculopathies of peripheral limbs or the retina are rare [100].

Pathophysiology

The major abnormality observed in aHUS is a form of TMA that primarily affects the blood vessels of the kidneys. Histologically, this process is indistinguishable from the pathology documented in other forms of HUS. It remains unclear why such a diverse array of HUS subtypes all exhibit exquisite tropism for the kidney microvasculature: it may be due at least in part to the stress generated by the unique rheological properties of glomerular capillaries (high flow, high pressure). TMA lesions are initiated when a severe injury to glomerular capillaries of multiple vascular beds at once triggers repair mechanisms that lead to a local prothrombotic environment.

The trapping of platelets in the microthrombi explains thrombocytopenia. Microangiopathic anemia is due to the shearing of erythrocytes as they transit through the partially obstructed glomerular capillaries. The initiating factor in most (but not all) patients with aHUS is related to abnormal activity of the alternative pathway (AP) of the complement system, which in turn leads to a strong immune attack of glomerular endothelial cells. In contrast, the pathophysiology of most complement-independent forms of aHUS is poorly understood. Discoveries related to the genetic or autoimmune causes of aHUS have been invaluable to devise therapies that address the underlying pathophysiology.

Genetics

Most reviews on aHUS suggest that ~60% of patients diagnosed with aHUS have a pathogenic genotype [104]. Of interest, a recent study on more than 3100 aHUS patients from 6 major centers reported that only ~40% had a mutation in one of the major aHUS genes [105]. The true yield of current genetic testing panels for aHUS is likely between those two figures. There is no doubt that clinicians should consider including a genetic testing panel in their early management care plan. Indeed, genetic testing results will help predict the overall prognosis and response to established therapies, determine the optimal timing of eculizumab discontinuation, and plan for suitable pre- and post-transplant precautions [68]. Below, we first discuss the major forms of genetic aHUS associated with complement abnormalities, followed by a presentation of the most salient genetic aHUS subtypes that cause pathology independent of the complement.

Genetics Forms of Complement-Mediated aHUS

Pathogenic variants in a host of complement genes are often identified when investigating patients with aHUS (e.g., *CFH*, *CFI*, *CFB*, *C3*, *MCP*, *CFHR5*). The mutation identified conspires to enhance the activity of the alternative complement cascade. As such, genes encoding negative regulators of the complement are expected to harbor variants that cause loss-of-function (*CFH*, *CFI, MCP*), whereas positive regulators have gain-of-function variants (*C3, CFB*).

The genetics of complement-driven aHUS is very complex because it includes allelic heterogeneity (different variants in the same gene lead to similar phenotype), locus heterogeneity (variants in different genes lead to similar phenotype), and mode-of-inheritance heterogeneity (heterozygous or homozygous variants in the same gene can cause the same disease) [106]. Importantly, three genes also exhibit phenotypic heterogeneity since variants in *CFH*, *C3*, or *CFHR5* can cause not only aHUS but also C3 glomerulopathy (a phenotypically distinct disease). Also, most of these conditions exhibit incomplete penetrance (a given variant is observed in affected and non-affected relatives) and/or variable expressivity (all relatives with a given variant are affected by the same disease with a wide spectrum of severity) [106].

CFH-Associated aHUS

Complement factor H is one of the major negative regulators of the alternative complement system: it controls the formation of C3b in the fluid phase (Fig. 2) [107]. The discovery of mutations in *CFH* provided the first proof of the existence of a genetic form of aHUS [108]. Mutations in *CFH* are the most frequent pathogenic genotype identified in aHUS patients: it occurs in ~40% of aHUS patients with a confirmed molecular diagnosis (Fig. 4) [105]. Patients with homozygous *CFH* mutations typically present in infancy with severe disease whereas patients with heterozygous mutations present at any age with milder forms of aHUS [109]. All pathogenic *CFH* genotypes either lead to decreased production of CFH proteins or to the production of a nonfunctional form of CFH [88].

MCP-Associated aHUS

Membrane cofactor protein (MCP, also known as CD46) is a cell surface negative complement regulator: it functions as a cofactor for FI, thereby assisting with C3b degradation (Fig. 2) [107]. The link between *MCP* mutations and aHUS was first established in 2003, with ~75% of kindreds exhibiting incomplete penetrance in the context of a heterozygous mutation [110, 111]. More severe disease is associated with recessive *MCP* mutations or heterozygous *MCP* mutations accompanied by another mutation in one of the complement genes [112]. Pathogenic mutations in *MCP* are reported in ~16% of aHUS patients with a confirmed molecular diagnosis (Fig. 4) [105]. They lead to either decreased amounts of MCP proteins on the cell surface or to impaired ability to regulate complements at the surface of endothelial cells [96]. On that basis, the risk of post-transplant recurrence would be predicted to be low: the higher observed rates are due to the repopulation of the allograft vasculature with recipient endothelial cells, a phenomenon known as endothelial microchimerism [113].

CFI-Associated aHUS

Factor I (FI) is the third major negative regulator of the complement system (Fig. 2) [107]. It was therefore not surprising when two teams reported that *CFI* was frequently mutated in patients with aHUS [114, 115]. Incomplete penetrance and variable expressivity are the norms for *CFI*-associated aHUS. Recent data suggest that ~10–15% of aHUS patients have a heterozygous *CFI* mutation (Fig. 4) [105]. Like in *CFH*-associated aHUS, a *CFI* mutation leads to low CFI protein levels or reduced enzymatic activity, thereby causing higher activation of the alternate pathway [116].

C3-Associated aHUS

Complement component 3 (C3) is central to the alternative complement cascade as it is the substrate required to generate the bioactive fragments C3b and C3a (Fig. 2) [107]. Pathogenic heterozygous *C3* mutations, which were first reported in 2008, resulting in a gain-of-function phenotype by preventing the degradation of C3b (e.g., via reduced binding to CFH or MCP) [117]. In some rare cases, the gain-of-function phenotype is due to enhanced C3 binding to CFB [118]. About 10–15% of aHUS patients harbor C3 mutations (Fig. 4) [105, p. 201].

CFB-Associated aHUS

Cleavage of factor B (FB) by factor D (FD) is necessary for the production of C3 convertase, C3bBb (Fig. 2) [107]. On that basis, gain-of-function *CFB* mutations were predicted to cause disease, and this is precisely what was found: heterozygous *CFB* mutations caused either enhanced formation or delayed the decay of C3 convertases [119]. It is now clear that this is a rare form of aHUS, accounting for ~2–4% of all confirmed cases of aHUS (Fig. 4) [105, p. 201]. FB-associated aHUS are generally fully penetrant [120], but kindreds consistent with incomplete penetrance are also described [119].

Thrombomodulin-Associated aHUS

Although previously mostly known as a cofactor for thrombin, preventing blood coagulation [121], thrombomodulin (THBD) was subsequently demonstrated to regulate FI-dependent degradation of C3b [122]. These studies were triggered by the discovery of an association between heterozygous *THBD* mutations and aHUS more than 10 years ago [122]: *THBD* mutations now account for ~1–2% of aHUS patients (Fig. 4) [105]. There is also some evidence linking *THBD* mutation with increased risks of venous thrombosis or myocardial infarction [123]. The pathophysiology of this condition may be due to the combined disturbances of the complement system and the coagulation cascade. More data is needed to make robust inferences regarding long-term outcomes or the impact of complement-based therapies.

Genetic Forms of aHUS Unrelated to Complement

Mutations in genes, that are not a component of the alternate pathway, have been reported to cause aHUS. These include cobalamin C (*cblC*) [124], diacylglycerol kinase epsilon (*DGKE*) [125], and plasminogen (*PLG*) [126]. There is currently no clear evidence that therapies targeting the alternate pathway are effective for the above conditions unless patients also carry a mutation in one of the complement genes. It also is unclear if these genes play a role through their interaction with complement or whether a coagulation protein-specific mechanism is involved. It is possible that additional research will uncover hitherto unknown physiologic roles for the products of these genes in the regulation of the complement system.

Cobalamin C Deficiency-Associated aHUS

With only ~40 cases reported since 1992, cobalamin C (cblC) deficiency-associated HUS is a rare form of TMA [127, 128]. This autosomal recessive condition is caused by mutations in the gene encoding for methylmalonic aciduria and homocystinuria type C protein (*MMACHC*) [124, 129]. Patients with this condition typically present as neonates with failure to thrive, neurologic symptoms, and features of TMA [127]. A subset of patients present as teens [127], and adult-onset is unusual [130]. High mortality has been reported for infantile *clbC*-associated HUS [127, 131]. The diagnostic hallmarks are hyperhomocysteinemia, low blood methionine, and methylmalonic aciduria [132]. Patients are usually treated with a combination of hydroxocobalamin, folinic acid, and betaine [133]. The impact of this cocktail on neurologic and vascular pathology is variable.

Several mechanisms are proposed to explain the TMA lesions, including endothelial damage induced by hyperhomocysteinemia or impairment of nitric oxide-dependent inhibition of platelet aggregation [127, 132].

DGKE-Associated aHUS

First reported in 2013 [125], *DGKE*-associated aHUS is one of the most common forms of aHUS diagnosed in children less than 2 years old. Patients with this condition experience TMA recurrences until 5 years of age (usually triggered by infection), have increasingly worse hypertension and proteinuria with age, and develop ESKD between the ages of 10 and 25 [134, 135]. The clinical features do not improve with eculizumab, and there are no documented cases of post-transplant aHUS recurrence. There are now more than 60 patients diagnosed with this ultra-rare condition [134, 135]; patients with DGKE nephropathy account for ~4–5% of all aHUS patients (Fig. 4) [105]. Patients with recessive pathogenic genotypes show almost complete deficiency of DGKE activity [134]. While most patients have clear loss-of-function *DGKE* variants (nonsense, frameshift, splice site), about ~30% have missense *DGKE* variants [134]. About 5–10% of patients with clearly pathogenic *DGKE* genotypes present with an active glomerulopathy, but without findings of TMA [136], a phenomenon that remains unexplained. While the exact pathophysiology remains elusive, it appears that *DGKE* deficiency causes endothelial cell activation [137], perhaps by interfering with prostaglandin E2 production [138] or signaling by vascular endothelial factor receptor-2 [139].

Plasminogen-Associated aHUS

Since thrombosis is central to TMA, a group of patients with undiagnosed aHUS were screened for mutations in all major genes implicated in the coagulation pathway using targeted exome sequencing [126]. The most notable finding was that 3/36 patients had heterozygous plasminogen (*PLG*) variants, all of which were known to cause *PLG* deficiency in the homozygous state. A recent study revealed that ~1% of patients with aHUS have a *PLG* variant (Fig. 4) [105]. In principle, *PLG* deficiency could reduce thrombi degradation, leading to a prothrombotic state [140]. The same question was the subject of intense investigations 20 years ago for patients with venous thromboembolism: the overwhelming conclusion was that heterozygous *PLG* deficiency is a benign condition [141]. It remains to be established whether *PLG* variants are causal, or act as a risk factor, for aHUS.

Autoimmune Forms of aHUS

The discovery that mutations in many complement genes caused aHUS led to the hypothesis that autoantibodies directed against the complement proteins could perhaps trigger an autoimmune form of aHUS [142, 143]. In 2005, investigators provided the first demonstration that anti-FH autoantibodies were present in a subset of patients with aHUS, resulting in an acquired functional FH deficiency [144]. The antibodies either reduced serum FH levels or interfered with the binding of FH to C3 convertase [144–146]. Of interest, most patients with anti-FH autoantibodies have homozygous deletions of *CFHR1* and *CFHR3*, suggesting that the gene deletions may play a role in the development of autoantibodies [147–149]. The functions of FHR proteins and their role in autoantibody production is not well understood [150]. In addition, in aHUS patients, anti-FI autoantibodies were found which also cause activation of the complement system [151]. Anti-FH and anti-FI autoantibodies typically account for ~10–25% and ~2% of aHUS cases, respectively, except in India, where anti-FH autoantibodies are the chief cause of aHUS and occur in ~55% of aHUS patients [152], and anti-FI autoantibodies are found in ~30% of patients [153]. Interestingly, anti-C3 autoantibodies enhancing the activity of the (alternative pathway) C3 convertase upon binding (so-called C3 nephritic factor, C3NeF) are found in patients with C3G, but have not been reported as a cause of aHUS [154].

Patients with autoimmune HUS often show a relapsing course [152, 155]. Higher titers of FH antibodies have been associated with a high risk of relapse [152]. Treatment guidelines recommend either prompt initiation of therapy with PLEX and immunosuppression or eculizumab with immunosuppression [82, 93, 156]. Treatment should be guided by FH antibody titers and once undetectable for at least 6 months KDIGO suggests that treatment could be discontinued [93]. There has been no study to compare the efficacy of eculizumab vs. PLEX. Several case reports report successful use of eculizumab either after failed PLEX or as initial treatment [157, 158]. Five to seven PLEX sessions achieve 80% reduction in antibody titers [159]. Immunosuppressive therapy mainly includes steroids plus either mycophenolate mofetil, cyclophosphamide, or rituximab [155, 156, 160, 161]. There is no data available that favors one over the other, and the choice of immunosuppression will depend on treatment availability and how comfortable clinicians feel with either treatment option. Overall outcome has been good for patients with FH antibody-associated aHUS if treated with PLEX/eculizumab and immunosuppression. However, kidney failure has been reported in about 10–25%. Late initiation of treatment is associated with worse outcomes [152, 157, 160].

Diagnosis

The diagnosis of aHUS is clinical and based on clinical features and absence of other disorders. There are no specific labs that will aid in the diagnosis of aHUS acutely. As previously mentioned, the presentation with the triad is nonspecific. It is necessary to exclude TTP by ruling out a functional abnormality or mutations in *ADAMTS13*. It is also critical to rule out STEC-associated HUS, especially if the patient presents with diarrhea. Circulating C3 and C4 complement assays are not useful since only 30–40% of patients with aHUS [85, 89, 162, 163] will have abnormal levels. Decreased plasma levels of FH or FI are seen in ~50% and ~30% of patients with significant variations in *CFH* or *CFI*, respectively [85, 164]; assays to quantify these proteins are not widely available. It is important to emphasize that normal serum C3 levels do not rule out aHUS, and that mutant *CFH* or *CFI* may be associated with normal plasma levels. The presence of high soluble C5b9 levels in serum is perhaps a useful indicator of disease activity but has drawbacks (high cost, not widely available, lack of assay standardization) [165].

While genetic testing with current aHUS gene panels is invaluable to distinguish aHUS subtypes, a detailed report is available only after a few weeks. Furthermore, in ~40–60% of cases, a significant variation might not be detected [85, 89, 162, 163]. Genetic studies, however, are necessary to guide subsequent treatment and are useful for long-term management. Current aHUS genetic testing panels include *CFH*, *CFI*, *MCP*, *C3*, *CFB*, *CFHR5*, *THBD*, and *DGKE*. The detection of hybrid *CFH* genes and copy-number variations in *CFH* or *CFHR1–5* are done using multiplex ligation-dependent probe amplification (MLPA) [166]. Investigations for anti-FH antibodies should also be performed. Patients with findings consistent with cobalamin C deficiency should undergo detailed biochemical characterization and genetic testing for *MMACHC* mutations (Fig. 3).

Management of aHUS

As with all renal diseases, management should include optimal management of hypertension, proteinuria, and attention to the effects of acute or chronic kidney disease in the child. Historically, the mainstay of treatment of aHUS has been the institution of plasma therapy (either infusion or exchange). The theory behind the use of either of these interventions hinges on the concept of either removing an abnormally functioning protein or replacing a deficient one. Treatment efficacy for plasma was based on the initial reports of success in FH-related diseases [89]. Consensus guidelines have suggested that plasma exchange be instituted as soon as possible after the diagnosis of aHUS, using 50–100 mL/kg of plasma per session [167]. The recommendation was for PE to be performed daily and the dose titrated to

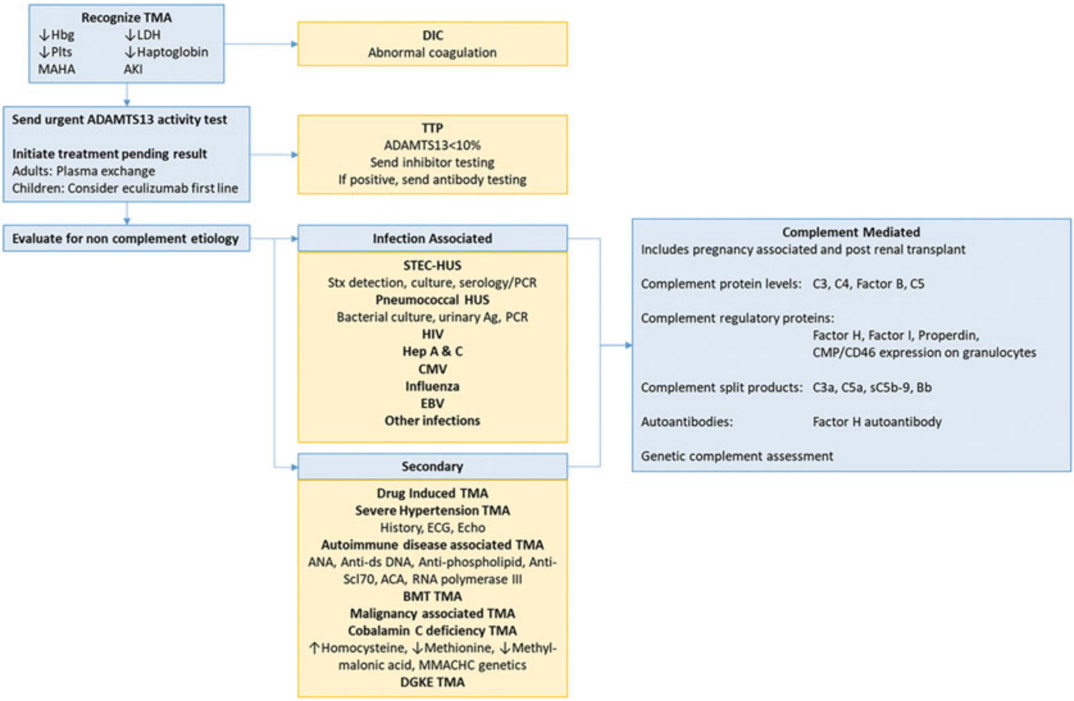
Recognize TMA
↓Hbg ↓LDH
↓Plts ↓Haptoglobin
MAHA AKI
DIC
Abnormal coagulation
Send urgent ADAMTS13 activity test
Initiate treatment pending result
Adults: Plasma exchange
Children: Consider eculizumab first line
TTP
ADAMTS13<10%
Send inhibitor testing
If positive, send antibody testing
Evaluate for non complement etiology
Infection Associated
STEC-HUS
Stx detection, culture, serology/PCR
Pneumococcal HUS
Bacterial culture, urinary Ag, PCR
HIV
Hep A & C
CMV
Influenza
EBV
Other infections
Secondary
Drug Induced TMA
Severe Hypertension TMA
History, ECG, Echo
Autoimmune disease associated TMA
ANA, Anti-ds DNA, Anti-phospholipid, Anti-Scl70, ACA, RNA polymerase III
BMT TMA
Malignancy associated TMA
Cobalamin C deficiency TMA
↑Homocysteine, ↓Methionine, ↓Methyl-malonic acid, MMACHC genetics
DGKE TMA
Complement Mediated
Includes pregnancy associated and post renal transplant
Complement protein levels: C3, C4, Factor B, C5
Complement regulatory proteins: Factor H, Factor I, Properdin, CMP/CD46 expression on granulocytes
Complement split products: C3a, C5a, sC5b-9, Bb
Autoantibodies: Factor H autoantibody
Genetic complement assessment

Fig. 3 Diagnostic algorithm for thrombotic microangiopathy (TMA). Diagnosis of a complement-mediated disease starts with recognition of thrombotic microangiopathy (TMA), which includes thrombocytopenia, microangiopathic hemolytic anemia (MAHA), and organ injury, including acute kidney injury (AKI). TMA can be differentiated from disseminated intravascular coagulation (DIC) by abnormal coagulation times. ADAMTS13 should be sent urgently to evaluate for thrombotic thrombocytopenic purpura (TTP). Evaluation for infection-associated and secondary TMAs should be investigated in tandem with complement-mediated TMA, and therapy should be initiated promptly while workup is underway. *BMT* bone marrow transplant-associated, *ECG* electrocardiogram, *STEC-HUS* Shiga toxin-producing *Escherichia coli* hemolytic uremic syndrome [65, 68, 82, 92, 93]

control hemolysis. Once hemolysis was controlled, the frequency of PE was reduced and then discontinued.

Noris et al. reported that plasma therapy resulted in complete or partial remission in 78% of aHUS episodes in children and 53% in adults. Looking closer at this single cohort however revealed that despite hematologic improvements, 48% of children and 67% of adults either died or reached end-stage renal disease (ESRD) at follow-up [89]. It has become apparent that plasma therapy may be reasonably successful at improving hematologic parameters but is not uniformly successful at facilitating renal health. The lack of availability of an alternative therapeutic regimen in some settings and comfort with the delivery of this care ensures that plasma therapy will continue to be used per local custom.

Given the current understanding about the pathophysiology of aHUS, complement blockade has become the gold standard for the treatment of this disease. Eculizumab, a monoclonal humanized anti-C5 antibody, prevents C5 cleavage and formation of both C5a and C5b-9. Therefore, eculizumab therapy can reduce anaphylatoxin-induced inflammation and limit C5b-9 prothrombotic consequences of complement activation. Trial data supports the efficacy of weight-based dosing of this agent [168] in the treatment of aHUS, and eculizumab is now approved for the treatment of aHUS both in the United States and in Europe (Table 1). An eculizumab concentration of 50–100 μg/mL will typically produce complete complement blockade in patients with aHUS [165, 169]. While drug level monitoring is not routinely practiced, it has been used to tailor medication dosing in patients with severe or atypical diseases [170, 171], and to reduce unnecessary drug utilization [172].

Whenever possible, eculizumab should be started as rapidly as possible in all children with aHUS at a standard dose. Vaccination status should be evaluated for all meningococcal strains and for *Streptococcus pneumoniae* and *Hemophilus influenzae*; these vaccinations are completed as soon as possible, and subsequently kept up to date during prolonged complement blockade. If vaccination is not possible or until 15 days after it is completed, antibiotic prophylaxis against meningococcal infection must be administered. Moreover, in children <5 years of age, in immunosuppressed individuals, and in those who live in communities, low-dose prophylactic antibiotics may be advisable even with a complete vaccination schedule. Patients receiving anti-C5 treatment should be advised of the risk of meningococcal infection, albeit low, instructed on early identification of warning signs and symptoms, and provided with an information card to carry at all times allowing for prompt recognition of their disease and treatment upon hospital admittance [82].

A long-acting form of eculizumab, ravulizumab, has been assessed both in paroxysmal nocturnal hematuria and in aHUS and has been shown to effectively control the disease while allowing less frequent infusions both in adults with aHUS [173] and in children previously treated with eculizumab [174].

Treatment with anti-C5 (eculizumab, ravulizumab) or with other complement-blocking agents that are currently being evaluated is not a disease-modifying approach, as it does not eliminate the underlying complement dysregulation in patients with aHUS. Barring forms driven by anti-FH antibodies, where standard immunosuppression can lower these antibody titers to the extent that it eliminates the driver of disease, and forms due to *DGKE* mutations which are complement-independent, in all other forms of aHUS due to alternative pathway of complement dysregulations, interrupting this treatment entails a variable degree of risk of disease recurrence. Therefore, if, how and when to discontinue anti-complement therapy is a difficult issue that does not have a single answer. Elements that must be considered are the presence and type of complement mutation, which determines the degree of risk, together with the morbidity of prolonged repeated intravenous infusions, risk of meningococcal infection, and especially in some settings the elevated costs of both the treatment and the infusions.

Special consideration is required for patients with aHUS triggered by autoantibodies. Many authors have reported that the risk of having a recurrent disease in this setting is based on the anti-FH antibody titers if there are no complement mutations identified. Therefore, it has been proposed that, in addition to anti-complement therapy at disease presentation, an acceptable treatment plan for antibody-mediated aHUS includes an antibody reduction strategy. This entails either use of plasma exchange or therapy with immunosuppressive medications (cyclophosphamide, rituximab, or mycophenolate mofetil) in order to reduce the antibody titer to a safe level. No trials are available to support this approach and the level of antibody titer may be lab dependent, therefore defining a “safe” threshold is challenging. Moreover, the length of eculizumab treatment required in these forms is still unclear.

Long-Term Complications

Prior to the introduction of anti-complement therapy, the prognosis for aHUS patients was poor. The 1-year mortality was reported to be as high as 6.7% in affected children, while end-stage kidney disease (ESKD) occurred in up to 16% [88]. At 5 years’ follow-up, 48% of children had reached ESKD.

Undoubtedly prognosis varies within genotype subgroups (Fig. 4). Individuals with *MCP* mutation carry the best prognosis [89]. Mutations in *CFH*, *CFI*, and *C3* show unsatisfactory outcomes. The first-episode mortality in children with *CFH* mutation was 30% [89] and evolution to ESKD in survivors was up to 33% in children [88]. Similarly, up to 60% of individuals with *CFI* or *C3* mutations reached ESKD or death at 5 years. At 5-year follow-up, 77% of patients with *CFH* mutations had developed either ESKD or death.

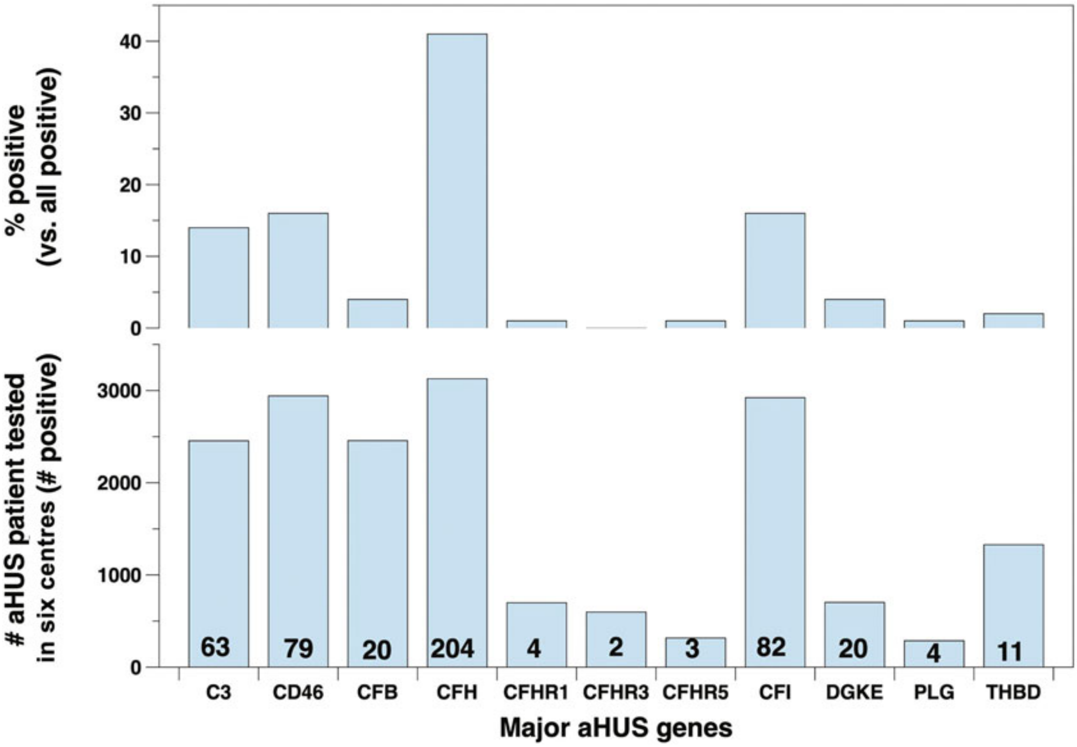

Fig. 4 Yield of genetic testing for a large cohort of patients with clinical features of aHUS. The data presented is from Osborne et al. [105], which collated genetic testing results from 3108 patients from six centers. The top panel illustrates the percentage of positive tests for given aHUS genes when compared to all positive tests. The bottom panel shows that the number of times each gene was tested is quite variable. It also shows how many patients harbored a variant in a given gene (numbers in bold).

Table 1 Weight-based dosing of eculizumab

Patient body weight	Induction regimen	Maintenance regimen
40 kg and over	900 mg weekly × four doses	1200 mg at week 5; then 1200 mg every 2 weeks
30 to <40 kg	600 mg weekly × two doses	900 mg at week 3; then 900 mg every 2 weeks
20 to <30 kg	600 mg weekly × two doses	600 mg at week 3; then 600 mg every 2 weeks
10 to <20 kg	600 mg weekly × one dose	300 mg at week 2; then 300 mg every 2 weeks
5 to <10 kg	300 mg weekly × one dose	300 mg at week 2; then 300 mg every 3 weeks

Thirty-three percent of children were likely to evolve to ESKD after the first episode. Up to 60% individuals with *CFI* or *C3* variations experienced ESKD or death. These statistics have significantly improved, in adults and children, with the advent of anti-complement therapy, with a maximum of 10–15% of patients in ESKD at 1-year follow-up irrespective of the mutation [175].

As mentioned, children with aHUS often have extrarenal symptoms. Long-term complications are similar to those seen in severe STEC HUS and include permanent renal injury and metabolic derangement, cardiac and gastrointestinal abnormalities, and occasionally residual neurologic disease.

Transplantation

Prior to the era of anti-complement therapy, the likelihood of aHUS recurrence in an allograft was entirely dependent on the causal mutation. Le Quintrec et al. [176] reported that aHUS patients had a 5-year graft survival of only 51% and this was associated with a 5-year mortality of 7%. *CFH*-related aHUS was most likely to recur in almost 90%, followed by *C3*, *CFB,* and *CFI* (50% recurrence risk). In contrast, post-transplant recurrence occurs in less than 10% of patients with *MCP* mutation. Post-transplant recurrence has also been observed in five patients with *DGKE* mutation. The recurrence risk is low in anti-FH antibody-associated HUS, as long as antibody titers are low.

It is generally accepted that aHUS patients may require some combination of plasma therapy and/or eculizumab prior to and post-transplant; however, protocols vary and prophylactic treatment should be based on the risk of recurrence. PE/PI for post-transplant recurrence generally failed to prevent graft loss. Therefore, prophylactic PE/PI (started just before transplantation) has been recommended [112] and should be used when anti-complement therapy is not available. Whenever possible, anti-complement therapy should be used to prevent recurrence starting just prior to renal transplantation in all forms of aHUS based on quantification of the risk of recurrence [93].

Briefly, high-risk patients are defined as those with a previous recurrence of aHUS on a renal allograft, or harboring pathogenic variants of *FH*, *C3,* or *FB* genes; moderate risk are patients with a negative complement screening or with a pathogenic variant in *FI* gene or with detectable circulating anti-FH antibodies; low-risk patients are those with undetectable circulating anti-FH antibodies who previously were positive and patients harboring *MCP* or *DGKE* isolated mutation. The use of prophylactic eculizumab starting immediately prior to transplant in patients with a moderate and high risk of recurrence was shown in a large retrospective study to significantly decrease aHUS recurrence and to significantly increase graft survival even compared to patients in whom recurrence was promptly treated with eculizumab, and it was also shown to have dramatically increased the numbers of renal transplants successfully performed in this patient population [177]. These data compellingly indicate that this is the correct strategy in aHUS patients with a moderate or high risk of recurrence.

Spectrum of Thrombotic Microangiopathy (TMA)

Introduction and Nomenclature

Thrombotic microangiopathy has been associated with several underlying conditions and historically this form of TMA was termed secondary HUS [82, 93]. A list of conditions that have been repeatedly associated with TMA are listed in Table 2. In the last two decades, researchers have started to

Table 2 Spectrum of disorders associated with thrombotic microangiopathy (TMA)

Pregnancy-associated TMA (pTMA)
Post hematopoietic stem cell transplant-associated TMA, or transplant TMA (TA-TMA)
TMA post solid organ transplantation
TMA associated with metabolic disease: Cobalamin C deficiency
TMA associated with systemic autoimmune diseases • Systemic lupus eythematosus • ANCA-associated vasculitis • Antiphospholipid syndrome • Systemic sclerosis • Mixed connective tissue disorder • Sjögren syndrome • Inflammatory bowel disease
TMA associated with drugs • Quinine • Anti-VEGF inhibitor • Chemotherapy such as gemcitabine, mitomycin, and cisplatin • CNI inhibitors
TMA associated with malignant hypertension
TMA associated with infections • Bacteria associated with a gastrointestinal infection, such as Shiga-toxin producing *E. coli*, *Shigella dysenteriae*, *campylobacter jejuni*, *Moraxella osloensis* • Viruses, such as cytomegalovirus, Epstein-Barr virus, varicella zoster virus, parvovirus B19, human immunodeficiency virus, influenza • Bacteria causing respiratory tract infections, such as *Bordetella pertussis*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, COVID-19 • Fungal infections in immunocompromised hosts
TMA associated with other renal diseases • C3 glomerulopathy • IgA nephropathy

investigate the underlying pathophysiology of TMA occurring in the context of another disease/drug and have found that some harbor an underlying complement defect and some do not. Some show signs of complement activation and some do not [178]. We currently think of TMA as a spectrum disease. On one end of the spectrum, you have atypical HUS with 70% of patients harboring a genetic/autoimmune alteration resulting in complement activation and high rates of disease recurrence and progression to kidney failure. On the other end, there are forms of TMA with no evidence for complement activation and therefore in need of a different treatment, mainly treatment of the underlying condition or discontinuation of the offending drug. Most TMA occurring in the context of another disease or drugs are somewhere in between the two extremes of the spectrum, and difficult to diagnose and treat. As the TMA spectrum includes a variety of coexisting diseases triggering TMA in different ways, we suggest to not use the phrase secondary TMA but rather use a descriptive term. In a patient with TMA developed in the setting post hematopoietic stem cell transplantation, we would call it TMA associated with HSCT or transplant TMA (TA-TMA, see Table 2). We will discuss in this chapter the current evidence of TMA spectrum diseases, the role of complement activation, and treatment recommendation.

Pathophysiology and Biomarkers

There are two ways how TMA can develop in the setting of a coexisting disease and/or associated with medications:

- The patient has an underlying complement disorder/genetic susceptibility and the disease/drug triggered the onset or the recurrence of the disease [178].
- The patient has no underlying complement disorder/genetic susceptibility but the disease/drug causes endothelial cell activation that resulted in endothelial cell injury and the clinical picture of TMA. Complement activation might result in secondary due to the endothelial cell injury; short-term complement blockade might be helpful in these cases.

Some conditions, for example, pregnancy, are more commonly a trigger event and the likelihood of finding an underlying complement abnormality is high. Other conditions such as malignant hypertension and drugs are more likely to result in endothelial cell injury and secondary complement activation. Some other conditions are less well defined.

We currently do not have reliable routinely available biomarkers that can help to distinguish genetic TMA from TMA associated with co-existing diseases. Low C3 can be observed in some patients with genetically driven HUS, but is not reliable [89, 179]. Measurement of complement activation products, for example, C3d and sC5b-9 might help determine systemic complement activation. However, similar to low C3 levels, half of the patients with atypical HUS have normal levels of complement activation products [179]. This might be explained by the fact that complement activation in TMA does not take place in the fluid-phase but on the surface of endothelial cells [180, 181]. Recently, Noris et al. have developed an ex-vivo test to determine complement activation. In this assay, they add serum of patients on either activated or not-activated endothelial cells and measure C5b-9 deposition. In active disease, high C5b-9 deposition was seen on activated and non-activated endothelial cells, compared to only on activated cells during remission. This was true for samples from patients with aHUS as well as secondary TMAs. Interestingly, no C5b-9 deposition was detected in patients on eculizumab treatment [182]. Another group corroborated the results, showing massive ex-vivo C5b-9 activation in 60% of patients with TMA and coexisting diseases [183].

Table 3 lists the investigations in patients with TMA. Treatment decisions are based on clinical features and severity of the disease, and reports on complement biomarkers.

Clinical Presentation and Outcome

Clinical presentation includes microangiopathic hemolytic anemia, thrombocytopenia, and kidney disease, which can manifest as elevated creatinine, proteinuria/nephrotic syndrome or nephritis,

Table 3 Workup in patients with thrombotic microangiopathy (TMA)

Investigation	Remarks
TMA markers • Hemoglobin, leukocyte, and platelet counts; schistocytes • Hemolysis: LDH, haptoglobin, bilirubin, Coombs test • Kidney disease: creatinine, urea, urine protein	Baseline investigations; also required for monitoring
Determine underlying etiology **Infections** • Shiga-toxin: stool PCR, serology • Pneumococcal: blood, CSF culture • Infections: serology, culture (influenza, HIV, HepB/C) **Autoimmune** • Antinuclear, antiphospholipid antibodies • Antineutrophil cytoplasmic, anti-double stranded DNA antibodies **Other kidney diseases** • C3 nephritic factor; glomerular basement membrane antibodies • Kidney biopsy **In woman** • Pregnancy test **Malignancy** • Complete blood counts, blood smear **TTP** • ADAMTS13 activity assay and inhibitor **Metabolic diseases** • Plasma homocysteine, methionine; urinary methylmalonic acid • Genetics if the above tests are abnormal, and metabolic consult **Disseminated intravascular coagulation** • Prothrombin time, partial thromboplastin time, fibrinogen	Investigation tailored to patient history and presentation ADAMTS13 testing needs to be sent prior to starting plasma exchanges
Determine complement activation • C3/C4; C3d, sC5b-9 • Alternate pathway (AP50) and classic pathway (CH50) testing • FH antibodies • Genetics: *CFH*, *CFI*, *MCP*, *C3*, *CFB*, *CFHR5*, *THBD*, *DGKE;* dosage analysis for hybrid *CFH* genes; copy-number variations in *CFH* or *CFHR*	Complement studies should be done prior to plasma therapy, or eculizumab initiation CH50 used for monitoring eculizumab efficacy Take DNA specimens prior to transfusion
Other organ manifestations • Liver function, cholestasis, amylase, lipase • Electrocardiogram, echocardiography • Neurological exam; brain imaging • If severe hypertension: evaluate for end-organ damage	Investigation tailored to the patient history and presentation. TMA is a systemic disease and every organ can be involved

or hypertension. Specific algorithms were published for pregnancy-associated TMA [46] and TMA post bone marrow/hematopoetic stem cell transplantation [184]. In general, adult patients with secondary TMA when compared to adult patients with aHUS have a lower risk of requiring dialysis or developing chronic kidney disease at initial presentation but have an increased risk of death (11% vs. 2%) [185]. Death was attributed to the underlying condition rather than TMA. Neurological involvement is reported in both aHUS and TMA spectrum disorders [185, 186]. Relapse was only noted in 1 out of 110 patients [185]. The risk of developing kidney failure was high with 37% of cases and another 40% progressed to CKD stage 3–4 within

2 years [185]. Worsening of kidney function might not all be due to TMA, as other factors like chemotherapy, transplantation and other kidney diseases may have contributed. Another cohort from Korea showed a 5-year survival rate of 60% in aHUS and TMA associated with coexisting diseases, with drug-induced and patients post-HSCT transplantation having the worst survival [186].

Treatment

Why is it important to distinguish whether the underlying pathophysiology of the TMA is related to complement activation or not? Treatment differs depending on the underlying cause leading to the TMA, but also important to know whether the patient has higher susceptibility for another event in the future. Furthermore, this might be relevant for other family members who could be affected.

The terminal complement inhibitor eculizumab has tremendously improved outcome in patients with aHUS [168]. Several case reports/small case series have indicated that terminal complement inhibition might be beneficial in secondary TMA. However, there have been no controlled studies in patients with TMA spectrum disease. One recent case series describes 110 patients with secondary TMA (infections, drugs, non-renal solid organ transplantation, malignancies, other glomerular diseases, or autoimmune diseases) receiving either plasma exchange (50%) or eculizumab (38%) [185]. Eculizumab was administered in the more severe cases and in one-quarter after plasma therapy had failed. Thirteen percent showed complete remission and two-thirds showed hematological remission and improved kidney function. However, the outcome was similar to patients that were not receiving eculizumab. Treatment was well tolerated, and no major side effects were reported [185]. In a smaller case series from Spain (29 patients, mainly drug-induced TMA or TMA associated with autoimmune disease), rapid improvement of hematological and renal parameters were observed in 70% of patients, most of them having failed plasma therapy before [187]. Initiation of eculizumab was delayed in most patients, and early (<7 days) initiation in patients with aHUS has been associated with improved long-term outcome [168]. Both these series had limitations, being retrospective in nature and including a very heterogeneous group, and therefore difficult for a recommendation [185, 187]. Furthermore, some TMA have more evidence for complement activation, for example, pregnancy and transplant-associated TMA and therefore better response to complement inhibition than others. In general, treatment in TMA with coexisting diseases should include therapy of the underlying condition or removal of the offending drug, if possible. Also strict control of blood pressure to avoid further endothelial cell injury is considered beneficial. Specific disorders are discussed below.

TMA Associated with Pregnancy

Incidence and Pathophysiology

Pregnancy has long been recognized as a condition that can precipitate an episode of TMA. The two main forms of TMA – TTP and HUS – have both been linked to pregnancy. TTP – both congenital and autoimmune form – is more common in cases with TMA developing during the second or third trimester of the pregnancy [68, 188]. The development of TTP during the last two trimesters of pregnancy is believed to result from increased VWF release and subsequent ADAMTS13 activation [68]. Complement-mediated TMA developed in the majority of the cases (75–80%) in the postpartum phase and was often associated with complement defects. Genetic screening of women with pregnancy-associated TMA showed an underlying genetic abnormality in 80% of the cases [188]. Additionally, patients had similar clinical presentation, rates of extra-renal complications and outcome, when compared to female patients with atypical HUS not related to pregnancy [189–191]. Pregnancy and nonpregnancy-related TMA share common genetic risk factors with a similar frequency (86% vs. 74%) and also share the same type of AP dysregulation, the most frequent abnormality being CFH mutations (40%) [189–191]. Pregnancy complications were common in women with pregnancy-associated TMA:

55% had pre-eclampsia and 33% HELLP syndrome [190]. Pregnancy TMA is considered to be mainly complement mediated.

Clinical Presentation and Diagnostic Approach

Clinical presentation is similar to other TMAs. Dialysis was required in 70% of patients. Diagnosis in patients with pregnancy-associated TMA is often delayed, due to the significant clinical overlap with HELLP syndrome and preeclampsia [190]. A recent consensus paper [46] suggested the following criteria to diagnose TMA in pregnancy:

- Platelet count $<100 \times 10^9$/L
- Hemoglobin level <10 g/dL
- LDH >1.5 upper limit of normal
- Undetectable serum haptoglobin
- Negative direct erythrocyte antiglobulin test
- Schistocytes on a blood smear (1), or features of TMA on kidney biopsy (2)

One should also consider the differential diagnoses and rule out HELLP syndrome and other possible underlying diseases such as SLE or catastrophic antiphospholipid syndrome (CAPS). An algorithm was published in the recent consensus paper [46]. Every patient should have an ADAMTS13 function and antibody test to rule out TTP. If this is negative a diagnosis of complement-mediated TMA can be made, treatment can be initiated and further workup is warranted [46, 82]. This workup includes genetics, autoantibodies, and complement activation markers as outlined in Ref. [68] (Table 3).

Treatment and Outcome

Outcome in patients with pregnancy-associated TMA is characterized – similar to aHUS – by high rates of kidney failure and disease recurrence [188, 190, 191]. ESKD was described in 50% over 7 years, with an additional 20% having reduced kidney function [189]. Relapses were observed in one-fourth of the patients. As pregnancy TMA has long been perceived as a complement-mediated disease several women were treated with eculizumab, which has significantly reduced rates of kidney failure [190, 191]. Eculizumab has been considered to be safe to use during pregnancy [190–194]. Plasma therapy was used in some patients with minimal response [188, 191]. Plasma exchange is the hallmark therapy for patients with pregnancy-associated TTP and often PLEX is started while ADAMTS13 testing is pending. Once ADAMTS13 is negative and other differential diagnoses have been excluded, patients should be commenced on anti-complement treatment. Treatment duration suggested by the recent consensus paper is a minimum of 6 months. If creatinine has been stable for >3 months, it can be considered to stop complement blockade [46].

Special Considerations

1. Women with aHUS can get pregnant but should be followed closely until 3–4 months post-delivery. The risk of recurrence during pregnancy is estimated as 20–25% [190, 193]. In the case of relapse during pregnancy prompt anti-complement therapy should be initiated to increase the chance of renal recovery and term live birth [46].
2. The miscarriage rate in patients with pregnancy-associated TMA is 9–14% (similar to the general population); however, one-third of babies show low birth weight [193, 194].
3. Patients who wish to get pregnant after a previous episode of pregnancy-associated TMA require counseling and close monitoring, as they have a high chance of relapse. Prophylactic anti-complement blockade may be considered in specific cases [193].

TMA Associated with Solid Organ Transplantation

Incidence and Pathophysiology

TMA post kidney transplantation can occur as a recurrence of aHUS or de-novo. TMA has also been described in patients post lung, heart, liver, intestinal, and pancreas transplantation [195]. TMA post solid organ transplantation is rare. In a recent meta-analysis, an incidence of 2–4% was reported in non-renal solid organ transplant patients and 1–14% in renal transplant recipients, with some TMA only evident in biopsies without hematological features [196]. The mechanism of endothelial cell dysfunction resulting in TMA post solid organ transplantation is thought to be multifactorial, including the use of cyclosporine and/or tacrolimus (calcineurin inhibitor, CNI) or other immunosuppressive drugs, infections, alloimmune reactions, rejection, and ischemia-reperfusion injury [197]. CNI is frequently used as part of the immunosuppressive regimen post transplant, and numerous reports describe the recovery of TMA with cessation of the drug. The first pediatric case of CNI-associated TMA was reported in 1999. Changing from cyclosporine to tacrolimus appeared to lead to renal recovery [198]. CNIs result in renal arteriolar vasoconstriction and increased platelet aggregation [197, 199]. In an attempt to limit the risk of calcineurin-inhibitor-associated nephrotoxicity, the use of the mTOR inhibitors sirolimus and everolimus have been proposed, however, de-novo TMA were also observed in patients with mTOR inhibitors [200]. In addition to blocking cell cycle progression and proliferation, mTOR inhibition also leads to decreased renal expression of vascular endothelial growth factor (VEGF) and death of endothelial progenitor cells; these latter anti-angiogenic effects are believed to contribute to the pathogenesis of TMA [201, 202]. Highest rates of TMA have been reported in patients on a combination of mTOR inhibitor and CNI [203]. Genetic studies revealed that 30% of patients with de-novo TMA post kidney transplantation harbor genetic variants in complement genes. This implicates a role for complement dysregulation, and surgery, infections, and immunosuppressive drugs could represent triggers that result in the manifestation of TMA in patients with genetic susceptibility [176]. The role of complement is less defined in non-kidney solid organ transplantation.

Clinical Presentation and Diagnostic Approach

There are no diagnostic criteria for TMA post solid-organ transplantation. In kidney allograft recipients, TMA can be restricted to the native kidneys, to the graft, or manifest systemically [195]. Thrombocytopenia and Coombs negative anemia are the main hematological symptoms, which can be difficult to interpret in liver transplants. Impaired renal function or failure is present in most cases; a biopsy is helpful to confirm the diagnosis [204]. One also needs to consider whether there is a chance that the patient's primary disease was aHUS and this is a recurrence rather than de-novo TMA. Antibody-mediated rejection (ABMR) results in endothelial cell injury and TMA can be a presenting feature [204]. TMA in ABMR has been associated with worse outcomes [205]. TMA post solid-organ transplantation, especially after kidney transplantation, warrants further workup as detailed in Table 3. A kidney biopsy is recommended, mainly to diagnose antibody-mediated rejection or other de-novo glomerulonephritis, such as lupus nephritis.

Treatment and Outcome

Prior to the use of complement inhibitors, systemic de-novo TMA was associated with poor outcome in adults: 40% graft loss rate and 50% mortality rate [200]. Similarly, TMA recurrence in the allograft was associated with high graft loss rates, but has much improved since using complement inhibition [177, 206]. Treatment of post-transplant de-novo TMA is often implemented in a stepwise fashion depending on what is believed the most likely etiology [204]. The first step has often been to discontinue or switch immunosuppressive drugs and that has frequently been shown to improve hematological parameters [204]. However, this has to be a careful consideration as decrease in immunosuppression might put patients at risk for rejection. Also, temporary or permanent discontinuation of CNI after the development of de novo TMA in the allograft has not been consistently shown to improve long-term graft outcomes [205]. Improved outcomes have been reported with PLEX and IVIG therapy in TMA associated with ABMR presumably as a result of the removal of HLA antibodies [205]. Similarly, to aHUS, several case reports show improved graft outcomes if patients were treated with CNI withdrawal and PLEX [207]. Recently, several case reports report the successful use of complement inhibition in patients with de-novo TMA post kidney and non-kidney transplantation [204, 208]. Of note, there are also some patients with ABMR with and without TMA that showed response to eculizumab [204]. There is no current recommendation on treatment, however, with previous reports showing that 30% have complement alterations we feel that complement inhibition should be promptly started in patients with systemic or biopsy-proven TMA and impaired kidney function, patients with a family history of TMA or unknown cause of kidney failure, as well as patients were switching immunosuppression or discontinuation is not feasible. In centers without access to complement targeted therapy, PLEX should be considered as an option.

TMA Associated with Hematopoietic Stem Cell Transplantation

Incidence and Pathophysiology

TMA associated with hematopoietic stem cell transplantation (HSCT) is recognized as a severe complication and significantly affects patients' well-being and transplantation outcome. It is mainly reported after allogeneic HSCT, but can also occur after autologous transplantation [209]. TMA was reported in 10–30% of pediatric patients post-HSCT, with one-third of them having severe disease [184]. TMA develops in the majority of patients within 100 days post transplant in median after 3 weeks [184]. Untreated patients with severe TMA (see criteria below) had a 1 year's survival of 17% and overall survival of 9%, milder cases had a risk of developing CKD [210]. Survival has much improved since complement target therapies became a cornerstone of treatment in severe cases. However, there remain challenges in diagnosing these patients and long-term renal involvement is common. Pathophysiology of TMA in patients post HSCT includes endothelial cell injury that results in a procoagulant state, activation of antigen-presenting cells, and activation of the complement system [211]. Risk factors include medications such as conditioning treatment and immunosuppression with calcineurin inhibitors, mTOR inhibitors, and infections [209]. A majority of patients with severe TMA (80%) show elevated markers of alternative and terminal complement activation [184], some even harbor variants of unknown significance or a homozygous *CFHR1/3* deletion [212].

Clinical Presentation and Diagnostic Approach

Hematological parameters are often not useful in patients post-HSCT and markers of kidney impairment, for example, hypertension and proteinuria, become important. Screening for TMA should therefore include regular CBC, blood smear, LDH, kidney function, urine dipstick, and blood pressure measurements. Nephrotic range proteinuria is described in half of the patients, 25% of patients required dialysis. Extrarenal complications are common, for example, pulmonary hypertension in 17%, severe pericardial effusion requiring drainage in 19%, lower GI bleeding in 35%, neurological symptoms in 33%, and occasionally multi-organ dysfunction syndrome. High-risk TMA according to the Cinicannti group is diagnosed if one of the following three conditions is present:

- TMA confirmed on kidney biopsy (A), OR
- Clinical diagnosis of TMA (5 out of 7 markers must be present and must include 6 and 7, B):
  1. LDH above normal for age
  2. Schistocytes on blood smear
  3. De novo thrombocytopenia or increased transfusion requirements
  4. De novo anemia or increased transfusion requirements
  5. Hypertension >99% for age (<18 years) or >140/90 mmHg (18 years of age)
  6. Proteinuria 30 mg/dL × 2; random urine protein/creatinine ratio >2 mg/mg
  7. Terminal complement activation (elevated plasma sC5b-9 above normal)

  Multi-organ dysfunction syndrome (at least 1 organ, C) and the presence of 5 of 7 criteria with at least one high-risk marker present (either 6 or 7 from criteria B).

A kidney biopsy is helpful in making the diagnosis, but often not feasible and then diagnosis is made on clinical basis using the above criteria. The workup is outlined in Table 3.

Treatment and Outcome

Eculizumab is considered the first-line treatment for patients with high-risk TMA post HSCT. Treatment differs from patients with aHUS as patients with TMA post-HSCT need more frequent dosing initially (sometimes as often as every 48 h) and can then be commenced on a rapid taper. Treatment monitoring using CH50 or eculizumab levels, if available, should be done regularly to assure the patient has adequate terminal complement blockade. If complement monitoring is not readily available, one has to closely monitor TMA markers (daily) and in patients with blood loss and frequent transfusions decreasing the interval between infusions should be considered in patients with inadequate response.

A meta-analysis of 116 patients from six studies indicated an overall response rate of 71%, complete remission in 32%, and survival in 52% [213]. A more recent report by the Cincinnati group shows a response rate of 64% and a survival rate of 66% 1-year post high-risk TMA [184]. The improved outcomes are most likely attributed to early initiation and efficient complement blockade in the first 2 weeks after diagnosis by dosing eculizumab to achieve trough levels ≥ 100 μg/mL and CH50 $<10\%$. Prognostic factors included a high pre-therapy sC5b-9 which was associated with a lower response rate, worse outcomes, and requiring more doses of eculizumab, reflecting massive complement activation and severity of the disease [184]. Patients with intestinal bleeding did worse. They also had higher eculizumab clearance and needed more doses, and a worse outcome. Even though patient survival has much improved, over 70% still have chronic kidney disease with lower GFR and persistent proteinuria [184]. Treatment of mild disease includes management of hypertension and decrease of immunosuppressive therapy, and treatment of infections.

TMA Associated with Autoimmune Diseases

TMA – HUS and TTP – seems to be overrepresented in systemic autoimmune diseases [214]. Systemic lupus erythematosus (SLE) and other connective tissue diseases are the most common diseases; however, others have been reported (Table 2) [178, 214]. TMA has also been described in patients with inflammatory bowel disease [215]. In the majority of cases, TMA occurs either concurrently with underlying autoimmune disease or after [214]. The difficulty is to diagnose TMA as clinical symptoms overlap significantly and biopsy is usually key to making the diagnosis. Treatment is mainly guided by the underlying disease, however, in refractory cases, PLEX and complement inhibition have been successfully attempted [216].

Systemic Lupus Erythematosus

Incidence and Pathophysiology

The prevalence of TMA in SLE patients is estimated at 1–9% with a higher prevalence in patients with lupus nephritis [216, 217]. TMA lesions in renal biopsies of patients with SLE were detected in a higher proportion (24%), however, most of them were lacking the clinical diagnosis of TMA [218]. TMA in majority occurs at the time of lupus onset or in a patient with known SLE [214]. Triggering factors include infection, pregnancy, lupus flare, and non-adherence [216]. In SLE, the formation of immune complexes activates the classical pathway and results in subsequent tissue inflammation [219]. Activation markers of alternate, classic, and terminal pathways were associated with disease activity in pediatric patients [220]. Complement defects are rare, but they are associated with increased risk for SLE [219]. In a large case-control study with 15,864 patients with SLE, the deletion of *CFHR3* and *CFHR1* was linked to the development of SLE [221] and in a different study, 6.7% of SLE patients had FH antibodies [222]. The pathogenesis of SLE-associated TMA remains unknown.

Clinical Presentation and Diagnostic Approach

Clinical symptoms for TMA in patients with SLE include thrombocytopenia as well as microangiopathic hemolytic anemia (DAT negative) with schistocytes and kidney disease. TMA should be suspected in SLE patients with rapidly progressive or persistent kidney disease (AKI, proteinuria, hematuria) and hypertension [216, 217]. Some adult case series report a 30–50% rate of dialysis in the acute phase [216, 217]. Of note, TMA should be suspected in patients on immunosuppressive therapy. Pathological evidence is key to identifying patients. Kidney biopsy commonly shows glomerular and vascular TMA including evidence of endothelial swelling, capillary and artery wall thickening, luminal fibrin thrombi, and erythrocyte fragmentation [214, 217].

Treatment and Outcome

Treatment of TMA in patients with SLE is most often guided by the treatment of the primary disease. Additionally, tight control of hypertension should be achieved to prevent further endothelial cell injury. Plasma exchange and complement inhibition with eculizumab has been reported as beneficial in some patients. Two retrospective analyses of 56 and 16 patients treated with PLEX indicate its benefit if administered seven or more times [217, 223]. There are several published cases that indicate the successful use of eculizumab, others show no difference in renal outcome (reviewed in Ref. [216]). One case report presents the successful use of intravenous soluble thrombomodulin [224]. In adults, the renal outcome is worse in patients with TMA [216, 225].

Antiphospholipid Syndrome (APS)

APS or life-threatening catastrophic APS (CAPS) is an autoimmune disease characterized by arterial and venous thrombi due to autoantibodies directed against phospholipids [226]. Kidney involvement (APS nephropathy, APSN) is common (71% during the course of disease), histopathologically often reflected by TMA [227]. APS is associated with recurrence post-transplant, especially presenting as TMA in the early phase after renal transplantation. Antiphospholipid antibodies lead to complement activation, likely via the classical pathway but increased C5a levels were also described [228, 229]. There have been case reports of patients with APS and TMA in biopsy (native and transplant kidneys) which were treated with eculizumab after failing PLEX treatment. Complement inhibition resulted in reversing clinical and histopathological findings of TMA [228].

Antineutrophil Cytoplasmic Antibodies (ANCA) Associated Vasculitis

TMA was detected in 10–15% of patients with ANCA-associated vasculitis [230]. TMA in these patients was associated with worse kidney disease and overall mortality [230]. Endothelial cell activation and injury in vasculitis are induced by activated neutrophils. This ANCA mediated response is amplified by alternate pathway activation on the endothelium, initiated by properdin and factor B released from activated neutrophils resulting in enhanced C5a production, closing an amplification loop of neutrophil activation [231]. Animal studies suggest a therapeutic role of anti-C5 blockade and clinical trials with avacopan, a small molecule antagonist of the human C5aR are promising [232]. The successful use of eculizumab was also described in a case report of ANCA-associated vasculitis [233].

TMA Associated with Cobalamin C Deficiency

Cobalamin C deficiency-associated HUS is a rare form of TMA and has been reported in fewer than 150 cases [89, 104, 110]. Inheritance is autosomal recessive and patients carry a mutation in the MMACHC (methylmalonic aciduria and homocystinuria type C protein) gene. TMA associated with Cobalamin C deficiency typically presents in the neonatal period with vomiting, poor sucking, failure to thrive, lethargy, hypotonia, thrombocytopenia, microangiopathic hemolytic anemia, and renal injury [234, 235]. Common renal involvement includes proteinuria, hematuria, and acute kidney injury including the need for short-term dialysis [127]. Case reports have also described the presentation of TMA in older children. Adult onset is extremely uncommon but reported [236].

Regardless of age at presentation, neurologic symptoms are prominent and also include cognitive impairment, ataxia, and psychosis.

Suggested mechanisms for TMA include endothelial damage induced by hyperhomocysteinemia, impairment of the nitric oxide-dependent inhibition of platelet aggregation, or induction of a procoagulant state of the endothelium leading to the formation of microthrombi [127]. Of note, a TMA-like picture has also been reported in patients with acquired cobalamin C deficiency [237].

A direct result of the cobalamin metabolism abnormalities, the biological hallmarks of Cbl C disease are hyperhomocysteinemia, low blood methionine, and methylmalonic aciduria. A finding of these laboratory abnormalities, particularly when no other diseases associated with TMA are identified, is enough to trigger a trial of therapy. Parenteral hydroxocobalamin combined with oral folinic acid and betaine is the recommended treatment for Cbl C disease [133].

The effectiveness of treatment regimens for patients with Cbl C disorder with or without TMA is variable. High mortality has been reported for infantile Cbl C-associated HUS [127, 131]. Improvements in biochemical, hematological, and growth parameters have been consistently reported in response to the treatment. Unfortunately, developmental delay, neurological involvement, and persistence or worsening of visual complications may persist. End-stage kidney disease is generally prevented by treatment.

TMA Associated with Drug Exposure

TMA from drug exposure may occur either from a toxin-mediated effect (dose-dependent or dose-independent) or it may occur from a dose-related, immunologic reaction. There are no reliable distinguishing features between drug-induced TMA and any of the other causes of TMA. Often the mechanism of disease pathology is not clearly defined. Renal failure is often present and ranges from mild to severe. Evidence supporting a direct causal role is limited for most reported associations. In a recent meta-analysis of the literature 78 drugs were described, however, the authors suggest that only 22 drugs had evidence supporting a causal relation to the TMA [238], the list is detailed in Table 2. Among the most reported agents that have been implicated include chemotherapeutic agents (gemcitabine, mitomycin, and cisplatin), calcineurin inhibitors (cyclosporine, tacrolimus), tyrosine kinase inhibitors, and vascular endothelial growth factor inhibitors [238–240]. Historically both ticlopidine and clopidogrel have also been associated with TMA but are infrequently used in the pediatric setting [241]. Quinine exposure is well reported to cause TMA in susceptible individuals [242, 243].

In general, treatment of TMA associated with drugs is guided by withdrawal of the potentially offending agents and monitoring for recovery. Plasma therapy has had unreliable effects and also the use of anti-complement therapy has been with mixed results [185, 187].

Quinine

Quinine exposure in children is highly unusual; however, it is worth mentioning quinine as the prototypical immune-mediated drug related to TMA. Initially recognized as recurrent kidney injury associated with hemolysis and thrombocytopenia after repeated quinine exposures [243], the mechanism for quinine-induced HUS is now reasonably well-defined. Antibody binding to multiple cells is facilitated by the presence of quinine – triggering disease [242, 244]. Quinine-dependent antibody binding may mediate thrombotic microangiopathy. Quinine-induced TMA often presents with sudden onset anuric renal failure. Few other systems are involved. In adults, supportive treatment including dialysis is often required. There is no evidence to support immune suppression or plasma therapy. Chronic kidney disease with hypertension is common in follow-up, and end-stage kidney disease is reported [245].

Chemotherapy

TMA occurring after exposure to chemotherapeutic agents is reported in both children and adults [238, 246]. Presentation includes not only the classic hematologic triad but also significant hypertension and renal failure. Extrarenal findings are much less common than in the other forms of TMA. Dose dependency was described for some drugs [247].

TMA following *mitomycin* exposure may be severe and lead to end-stage renal disease [248]. It often occurs 4–8 weeks after the last dose and after a cumulative dose of 40–60 mg. Mortality was reported to be as high as 70% [240]. Multiple cases of gemcitabine-associated HUS have been reported in adults [249]. Its incidence has been reported to range from 0.15% to 4% after treatment for 6–8 months with a total dose of 22.5 (±14) mg/dL. Kidney failure, hypertension, and proteinuria are common features [240]. Kidney biopsies have shown C3 deposition and successful use of eculizumab was described, indicating there might be a role for complement activation in its pathophysiology [250]. Cisplatin, an agent used for childhood tumors, has been reported in association with TMA [251]. Limited evidence suggests that cisplatin not only causes widespread endothelial damage but also may precipitate glomerular basement membrane and podocyte damage [252].

Additional chemotherapeutic agents that were reported to trigger TMA: bleomycin, doxorubicin, 5-fluorouracil, carboplatin, daunorubicin, dacarbazine, oxaliplatin, lomustine, vinblastine, tamoxifen, carmustine, tretinoin, and cytarabine [238]. Because TMA is also reported with cancer and independent of chemotherapeutic agents, ascribing the cause is difficult [240].

VEGF Inhibitors

Vascular endothelial growth factor (VEGF) inhibitors are the latest in chemotherapy agents with an association with TMA (sunitinib and bevacizumab) [239, 253, 254]. VEGF is produced by the podocytes and travels retrograde up the glomerular filter to maintain healthy endothelial cells. Lack of VEGF will result in changes of vascular tone, for example, vasoconstriction, which is also the proposed mechanism for hypertension induced by anti-VEGF therapy and often a leading symptom together with non-nephrotic range proteinuria [254]. For bevacizumab, the incidence of hypertension is reported as 25% and the incidence of proteinuria as high as 21–62% [239]. Biopsy often shows the hallmark features of TMA, including widening of the subendothelial spaces of glomerular capillaries, double contour of the GBM, mesangiolysis, effacement of foot processes, and endothelial swelling with overt thrombosis [254]. There have been some reports of worsening hypertension, proteinuria, and TMA even after intravitreal use of VEGF inhibitors, however, much less common compared to systemic use [255]. All patients receiving systemic anti-VEGF require close monitoring of their blood pressure and regular urine assessment for proteinuria. Improvement of proteinuria and hypertension was reported after the drug was discontinued [254].

TMA Associated with Infection

Besides STEC HUS, TMA has been reported to occur in the setting of several different bacterial, fungal, and viral infections. For a full list please see Table 2 (reviewed in Ref. [178], updated for Covid-19 infection [256] and fungal infection [257]). Besides EHEC- and Shigella-associated HUS in the developing world, pneumococcal HUS is the most frequent form of postinfectious HUS, accounting for around 5% of all childhood-onset HUS [258]. Atypical HUS onset or relapse is often triggered by even a simple infection or cold. If a TMA occurs in the setting of an infection, one has to ask themselves whether the infection could be a trigger and workup as shown in Table 3 should be performed.

If there are concerns for complement-mediated TMA (positive family history, recurrent disease, low C3, severe disease), one might consider treatment as in aHUS until work-up is back. In mild disease, one can treat the infection and monitor for resolution of symptoms of the TMA. It is more difficult to make this decision in severe infections. Additionally, sepsis is known to cause complement activation, including terminal pathway, and causes endothelial cell injury [259, 260]. TMA in septicemia and endocarditis [261] has also been described. However, before initiation of terminal complement inhibition one has to weigh the pros and cons, due to the risk of pneumococcal and meningococcal infections [262]. In a large pediatric cohort post hematopoietic stem cell transplantation, no increased rate of infections was seen in patients receiving eculizumab for TMA. Several bacterial, viral, and fungal infections were documented but none of them resulted in death. Of note, all patients received antibiotic prophylaxis while on eculizumab [184].

Streptococcus pneumoniae HUS

HUS may occur following an invasive infection with *Streptococcus pneumoniae*. With the emergence of the pneumococcal vaccine, the overall incidence of pneumococcal HUS has been decreasing in North America in recent years. The most common serotypes also changed over time, although serotype 19A – although covered in the 13 valent pneumococcal vaccine – is still the most common serotype [263]. The preceding *Streptococcus pneumoniae* infection is usually severe and invasive, and children may present with septicemia, meningitis, and/or pneumonia with empyema [3, 202, 205]. Children with pneumococcal HUS were generally younger, had more severe renal and hematological disease, and were more likely to require dialysis compared to patients with STEC HUS [264]. Patients with pneumococcal HUS also had a higher incidence of cortical necrosis and long-term renal and extrarenal complications compared to STEC HUS [264–266].

S. pneumoniae produces neuraminidase, which cleaves N-acetyl neuraminic acid from cell surfaces and thereby exposes the Thomsen–Friedenreich antigen (T-antigen) on cell surfaces. Naturally occurring antibodies bind the T-antigen leading to hemolysis (Coombs test positive), thrombosis and endothelial damage, and the clinical picture of TMA [91]. A similar mechanism was proposed for other neuraminidase-producing microbes such as influenza A [94]. *S. pneumoniae* activates the classical pathway, and early complement proteins (of all three pathways) are required for *S. pneumoniae* killing. Studies that looked into underlying complement defects found a higher amount of rare genetic variants in pneumococcal HUS patients, including the aHUS-risk polymorphisms in the *CFH-CFHR3-CFHR1* region [267, 268]. Based on these findings, we recommend complement genetic screening in patients who develop HUS in the context of *S. pneumoniae* infections, as well as to analyze aHUS-risk variants in these patients. Further research also points toward desialylated FH having a lower capacity to regulate complement activation on cellular surfaces [267].

Treatment focuses on treating the underlying infection. There have been case reports describing the use of eculizumab, especially in patients who showed signs of complement activation [269]. With the small number of published cases, it is difficult to give treatment recommendations.

TMA Associated with Malignant Hypertension

The link between malignant hypertension, nowadays mainly defined as a hypertensive emergency, and aHUS is the subject of an ongoing debate. Is the TMA complicating malignant hypertension due to an underlying complement defect or secondary to severe hypertension and secondary endothelial cell injury. Hypertensive emergencies complicated an aHUS diagnosis in 50% of adult patients [68]. Further 50% of those harbor underlying pathogenic variants, which is similar to patients with TMA not associated with severe hypertension. Their outcome was also comparable [68]. On the other hand, patients with malignant hypertension have only a small risk of developing TMA (3%) [270]. Indicators for the hypertension being more likely essential and not related to TMA are male, >45 years, previous history of hypertension, no clots on kidney biopsy, no need for renal replacement therapy, and quick recovery (<72 h) of the TMA with strict blood pressure control [68]. Clues that favor atypical HUS diagnosis are positive family history and recurring/persistent episodes of hematological features of TMA. Of note, some patients with malignant hypertension associated with TMA did not present with thrombocytopenia in the first place and diagnosis of TMA was only made by kidney biopsy [271]. A recent report of 26 Caucasian patients, with hypertensive emergency, and TMA confirmed by kidney biopsy, showed that patients presented with severe disease (neurological manifestations in 23% and dialysis in 65%) but profound systemic hemolysis was absent in 73%. Rare complement gene variants were present in 8 patients (31%). Sera from 17 of the 25 patients who were tested induced increased deposition of C5b-9 on endothelial cells in an in vitro assay compared with pooled normal human sera, regardless of the presence or absence of complement gene variants. Sixty-one percent of patients progressed to kidney failure within 3 months of diagnosis, five of seven patients responded at least partial response to eculizumab [271]. In pediatrics, TMA due to malignant hypertension is rare and a patient presenting with TMA and a hypertensive emergency should be considered for a patient with aHUS, and diagnostics as outlined in Table 3 should be sent. It is reasonable to start this patient on anti-complement therapy, especially if no rapid recovery after initiation of anti-hypertensive drugs is noted.

Conclusion

STEC HUS (▶ Chap. 24, "Infection-Related Hemolytic Uremic Syndrome (HUS)") and TTP (above) are defined by specific pathomechanisms – STEC HUS by Stx-producing *E. coli*, and TTP by the appearance of ULVWF multimers due to genetic or autoimmune defects in ADAMTS13. Different from these, aHUS and the spectrum of secondary TMAs are caused by injury to the vascular endothelium caused by complement dysregulation due to mutations or autoantibodies in primary aHUS, or enhancing endothelial lesions caused by a spectrum of underlying pathologies in secondary TMA [272]. Of note, both forms often follow preceding triggers [178]. A systematic and standardized approach to the diagnostic work-up of TMA patients is required to determine the correct diagnosis and, thus, identify the patients in whom the use of complement modulating medications is indicated. The documented treatment benefit of complement blockers in aHUS patients [168] holds major promise for future advancements in the outcomes and the quality of life of TMA patients.

Cross-References

▶ Infection-Related Hemolytic Uremic Syndrome (HUS)

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Infection-Related Hemolytic Uremic Syndrome (HUS)

24

Shoji Kagami, Müller Dominik, Michal Malina, and Akira Ashida

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Abstract

Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy (TMA) clinically defined by thrombocytopenia, nonimmune microangiopathic hemolytic anemia, and acute kidney injury. HUS is categorized as infection-related HUS, typically caused by Shiga toxin producing *Escherichia coli* (STEC), rarely by *Streptococcus pneumoniae*, as atypical HUS (aHUS), or as secondary HUS with a coexisting systemic disease. Recent data indicated the involvement of the complement system in the pathogenesis of infectious agent-induced HUS as well. This chapter reviews current concepts of infection-related HUS in the point of view regarding the classification, epidemiological findings, pathophysiological and clinical aspects of STEC, *Streptococcus pneumoniae*, and other infectious agent-associated HUS, and their diagnosis and management.

Keywords

Acute kidney injury · Hemolytic anemia · Thrombocytopenia · Thrombotic microangiopathy · *Escherichia coli* · STEC-HUS · *Streptococcus pneumoniae* · Pneumococcal HUS · aHUS · Complement system

Introduction and Classification

Thrombotic microangiopathies (TMA) is a heterogenous group of diseases where central pathophysiological principle is development of miniscule thrombi in microvasculature of organs including glomeruli. This is usually preceded by endothelial injury by various means. Though the final manifestation of TMA is similar, the etiology and pathophysiology are vastly different and have implications for management [1].

The greatest deal of cases is secondary to infections where the Shiga toxin producing *Escherichia coli* (STEC) is the most common. Hemolytic uremic syndrome (HUS) secondary to other infections is rare in comparison. The following classification summarizes infectious related causes of HUS (Table 1).

Epidemiology

Incidence of STEC Infection and STEC-HUS

HUS is characterized by the triad of hemolytic anemia, consumptive thrombocytopenia, and acute kidney injury. In children, STEC is the leading cause of HUS and accounts for approximately 90% of cases in Europe [2, 3] and 65% in Japan [4]. In 1983, *Escherichia coli* O157:H7 were detected in the stool of patients in an outbreak of hemorrhagic colitis associated with undercooked beef patty consumption [5], and Shiga toxin (Stx) was identified in the stool from the patients with HUS in the same year [6]. The incidence of STEC infection and a combination of STEC-HUS has been analyzed epidemiologically in various countries around the world. According to a global analysis based on articles and database

Table 1 Classification of infection-associated HUS

A. STEC caused HUS
B. *Streptococcus pneumoniae*-associated HUS
C. Other infectious agent-associated HUS Shigella Viral infection – influenza [1]

from 21 countries, the incidence of STEC infection has been estimated to be 43.1 cases per person-year from 1990 to 2012 [7]. This analysis estimated that 3,890 of 2,801,000 cases with STEC infection developed HUS annually. The age-specific incidence of STEC infection and STEC-HUS were estimated respectively as 28.8% (n = 809,000) and 41.9% (n = 1,630) in patients aged 0 to 4 years, 19.8% (n = 554,000) and 18.5% (n = 720) in patients aged 5 to 15 years, 34.8% (n = 974,000) and 26.0% (n = 1,010) in patients aged 16 to 59 years, and 16.6% (n = 464,000) and 13.6% (n = 530) in patients aged over 60 years [7]. These results suggested that both STEC infection and combined STEC-HUS occur more frequently in younger children than any other age group.

In the United States, the incidence of STEC infection increased gradually in 2015 to 1.5 cases/100,000 person-year from the beginning of 2000, and the incidence of STEC infection in children aged 1 to 4 years was the highest of all age groups for both O157 and non-O157 serogroups [8]. In the European Union, the incidence of STEC infection was reported to be 1.7 cases/100,000 person-year with children of ages 1 to 4 years having the highest incidence of 6.0 cases per 100,000 person-year among all age groups, followed by children aged below 1 year having 4.5 cases/100,000 person-year [9] (Table 2). In Japan, STEC infection is a notifiable disease, and its incidence including asymptomatic carrier is reported to be 2.7–3.5 cases/100,000 person-year since 2006 [10]. In 2019, 3,744 cases with STEC infection including 1,223 asymptomatic carriers were reported, with the highest incidence being in children aged 0–4 years, followed by those aged 5–9 years [11]. Therefore, it is clear from the epidemiological data from these countries that STEC infection is mainly contracted by the young children.

In Japan in 2019, 78 cases (3.1% of symptomatic patients with STEC infection) of the 3,744 cases of STEC infection developed STEC-HUS. The incidence of STEC-HUS among the symptomatic patients with STEC infection was the highest at 0–4 years of age (6.0%), followed by 5–9 years (5.9%), and 10–14 years (4.5%), indicating that the proportion of STEC-HUS patients among symptomatic STEC patients was higher in the younger age groups [11]. In the European Union, 8–12% of patients with STEC infection is reported to develop to HUS [9], among which the data from 2015 in France estimated the incidence rate of STEC-HUS to be 0.94 cases/100,000 person-year in children younger than 15 years of age. The estimated incidence of STEC-HUS in children aged less than <3 years (3.1 cases/100,000 person-year) was higher than those in children aged 11–14 years (0.2 cases/100,000 person-year) [12]. The epidemiological data of these countries confirmed the higher incidence of both STEC infection and STEC-HUS in

Table 2 Notification rates of confirmed STEC infection by age group and sex in the United States and EU/EEA

Age	United States in 2015 (Ref. [8])				EU/EEA in 2010–2012 (Ref. [9])		Age
	O157		Non-O157		STEC		
	Female	Male	Female	Male	Female	Male	
<1	0.87	2.21	1.96	2.11	4.7	4.6	<1
1–4	2.7	3.34	3.26	4.22	6.1	6.5	1–4
5–9	1.51	1.6	1.09	1.25	1.7	1.6	5–14
10–19	0.87	0.94	1.29	1.28	1.4	0.9	15–24
20–29	0.65	0.49	1.16	0.69	1.1	0.7	25–44
30–39	0.35	0.16	0.64	0.29			
40–49	0.23	0.14	0.41	0.22	1.0	0.7	45–64
50–59	0.29	0.15	0.42	0.18			
60–69	0.33	0.31	0.48	0.29	1.2	0.1	>65
70–79	0.36	0.33	0.5	0.46			
>80	0.31	0.24	0.56	0.26			
Overall	0.62	0.62	0.87	0.76	1.4	1.2	Overall

younger children, and it occurs more commonly in summer months.

Incidence of *Streptococcus pneumoniae*-Associated HUS

The *Streptococcus pneumoniae*-associated HUS (P-HUS) is reported to account for 4–5% of total HUS, and its annual incidence is estimated to be 0.015 to 0.06 per 100,000 children [2, 13, 14]. In addition, it has been reported that complicated pneumonia is strongly associated with P-HUS among various symptoms of invasive pneumococcal disease (IPD) including septicemia, meningitis, and complicated pneumonia. Although the incidence of IPD has decreased after the introduction of seven-valent pneumococcal conjugate vaccine (PCV7) [15], the incidence of P-HUS has increased rather than decreased [13], and the incidence of P-HUS among IPD patients has also increased [14]. A 19A serotype, not included in PCV7, has been frequently reported as a pneumococcal serotype identified as the cause of P-HUS. Since the 19A serotype is included in the 13-valent pneumococcal conjugate vaccine (PCV13), it is necessary to analyze whether PCV13 allows for a decreased incidence of P-HUS.

STEC-Associated HUS

Pathogenesis and Pathophysiology

Microbiology of *Escherichia coli*

The most common STEC serotype identified in cases of hemorrhagic enterocolitis and HUS in the United States, the European Union, and Japan is O157:H7. However, various serotypes of STEC have been identified to date. Non-O157 serotypes, such as the 2011 German O104:H4 outbreak and the O111 outbreak in Japan, can also cause an outbreak of hemorrhagic colitis and HUS. The 2011 German outbreak affected all age groups but predominantly females aged around 35 years and was identified as a multiresistant EAEC strain disseminated by contaminated sprouts [16]. This finding also challenges the concept of Gb3 surface expression as the only pathomechanism in STEC-HUS.

Recently, it has been reported that the ratio of O157 serogroups and of non-O157 serogroups has changed in STEC infections. In the United States, between 2000 and 2010, the incidence of O157 STEC infection decreased from 2.17 cases/100,000 person-year, and the incidence of non-O157 STEC infection increased from 0.12 cases/100,000 person/year, with both incidences reaching equivalent value of 0.95 cases/100,000 person-year [8]. In 2015, the incidence of O157 STEC infection and non-O157 STEC infection were 0.66 and 0.84 cases/100,000 person-year, respectively, and the incidence of non-O157 STEC infection exceeded the incidence of O157 infection [8] (Fig. 1). In the European Union, although O157 is the most common serotype in STEC infection and its rate was stable, it accounted for only 55% of the identified cases from 2007 to 2012 [9, 17] (Fig. 2). In Japan in 2019, the O157 serotype accounted for 54% of the identified cases [11]. The proportion of O157 serotype in STEC infection has tend to decrease in both countries having implications for diagnosis. The non-O157 strains are usually not detected by classical microbiological methods and require utilization of STEC PCR diagnostic [18].

Source of Infection

Cattle and its intestine are considered the primary reservoir of STEC. Deer, sheep, goats, horses, dogs, flies, and humans can also become vectors of STEC infection. Undercooked meat, contaminated by feces in abattoir, are common source of STEC infection. Feces-contaminated fruit and vegetables including fenugreek sprouts, unpasteurized dairy products, and contaminated water [19, 20] can become vehicles for transmission of STEC and were found as mean of infection in major outbreaks. In addition, petting zoos and close contact with farm animals have been identified as a source of STEC infections [19]. A meta-analysis of sporadic cases suggested the significance of ingestion of undercooked meat, contact with animals, and exposure in environments with feces of the animals

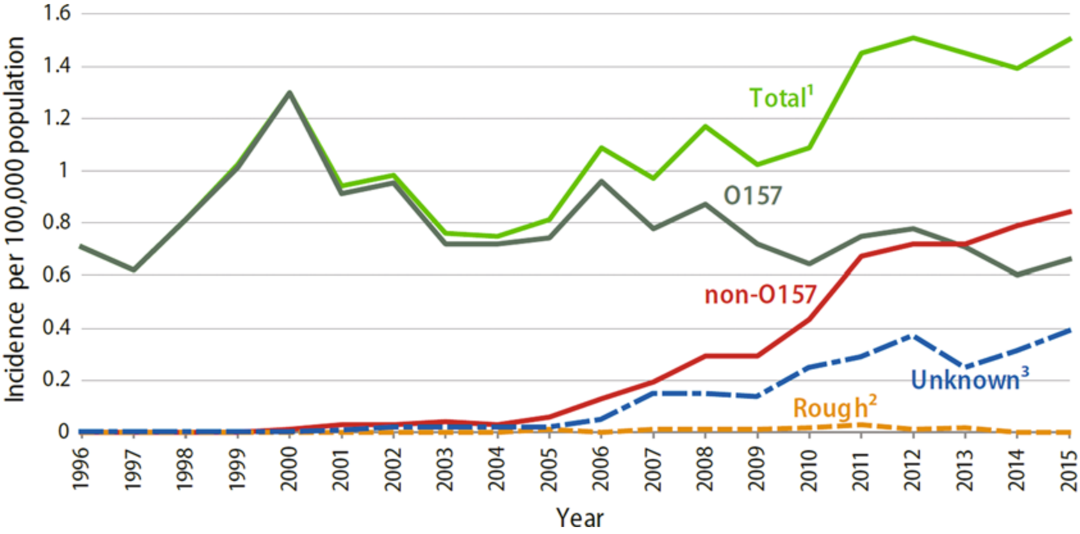


Fig. 1 Incidence rate of human STEC infection by serogroup and year, United States, 1996–2015. In the United States, the incidence of O157 infection decreased and the incidence of non-O157 infection increased since 2000. In 2015, the incidence of non-O157 STEC infection exceeded the incidence of O157 infection. (Reprinted with permission from reference [8])

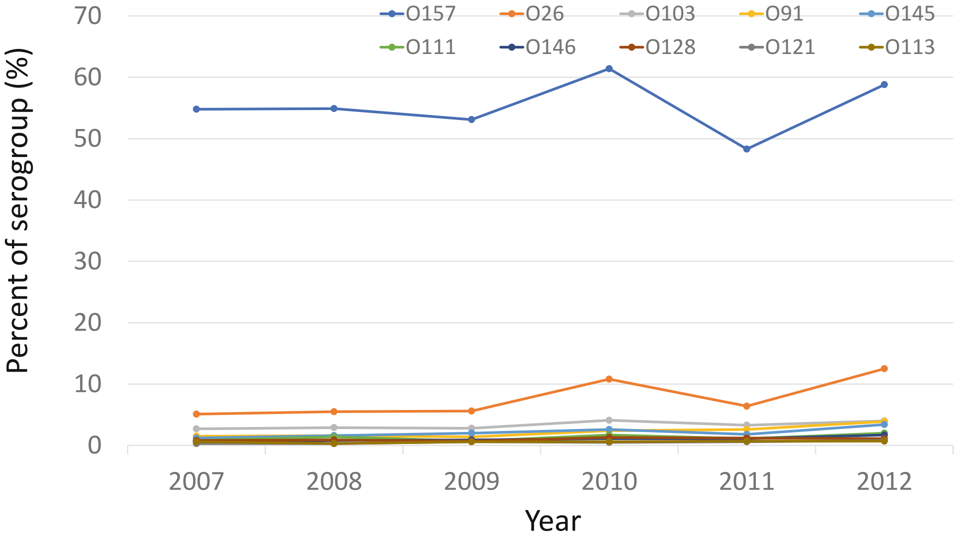


Fig. 2 Distribution of the ten most commonly reported STEC O-serogroups in confirmed cases as reported by EU/EEA countries, 2007–2012 (Reference [9, 17]). In EU/EEA, although O157 STEC infection is the most common and its incidence rate is stable, it accounted for only 55% of the identified cases from 2007 to 2012

as routes of transmission for STEC [21]. Another important transmission route of STEC is person-to-person transmission. Person-to-person transmission has been reported to be the second-most frequent transmission route of STEC after ingestion of undercooked or raw meat, and it is estimated that a complete suppression of transmission through person-to-person contact could reduce STEC infection by 15% [21]. As a result of the study for prevalence and serotypes of STEC in feces from about 473,000 healthy adults, including food handlers and person who worked in childcare facilities or nursing home, STEC was positive in 398 cases, and the incidence of asymptomatic carriers in healthy adults was estimated as 84.2 cases/100,000 person-year in Japan. Although the kind of serotype and characteristics of STEC detected from healthy adults differed from those detected from symptomatic patients, the presence of asymptomatic carriers in STEC suggests the possibility of person-to-person secondary infection as a route of transmission [10].

Diagnosis of STEC Infection

As confirmation of exclusion of STEC infection is essential for diagnosis of complement-associated HUS, selection of therapy, and estimation of prognosis, screening for STEC infection is recommended to be performed in all patients with HUS. STEC infection is led by a very low load of organisms (<100 organisms) [22], and excretion of STEC in the stool of patients generally lasts only a few days following the onset of diarrhea [12]. Therefore, stool samples should be obtained as soon as possible after the onset of diarrhea. If appropriate stool samples cannot be obtained, rectal swabs can also be collected [12]. STEC O157 is a non-sorbitol-fermenting representative strain of STEC that can be detected by the appearance of white colonies in culture on a sorbitol MacConkey agar plate. However, it should be noted that selective and differential detection using this method is not appropriate for the detection of non-O157 serotypes [12], which have been increasing in recent years. Once a STEC has been detected, its additional serotypes should be confirmed. To increase the identification rate of STEC infection, analysis of the genes encoding Stx (*stx1* and *stx2*) using PCR and detection of STEC and Stx using immunologic assays including ELISA should be conducted using incubated samples in enrichment culture simultaneously with the culture test for STEC detection [12]. In addition, the presence of anti-LPS antibodies in the serum of the patients who develop STEC infection, especially those who develop STEC-HUS, should be determined to carry out the serological diagnosis for the main STEC strain [12].

Characteristics of Stx

STEC produce two main types of Stx, known as Stx1 and Stx2 which were encoded *Stx1* and *Stx 2* genes. The strains of STEC can produce Stx1 and Stx2, alone or in combination. Each Stx is classified into subvariants, Stx1a, Stx1c, Stx1d, and Stx2a to Stx2k [23, 24]. The Stx2 is believed to be more virulent than Stx1, and Stx2-producing *Escherichia coli* is frequently detected in STEC-HUS patients either individually or with Stx1 production.

Stx is composed of a single subunit A and five subunit B. The subunit A is a biologically active subunit with 30 kDa of molecular weight and the subunit B is a cell recognition receptor binding subunit with a molecular weight of 7 kDa [22].

Mechanisms of Stx-Mediated Cell Injury (Fig. 3 [26])

Stx released by STEC in the intestine enters the circulation through the gastrointestinal epithelium. In the circulation, Stx is transferred to target organs such as the kidney, brain, and pancreas and express the specific Stx receptor, globotriaosylceramide 3 (Gb3) [26]. Gb3 is also known to be involved in the development of Fabry disease. By binding its subunit B to Gb3, Stx is internalized into the cells by endocytosis and transported in a retrograde manner to the endoplasmic reticulum. Thereafter, the subunit A translocates to the cytosol and interferes with ribosomal protein synthesis through the depurination of adenosine at 28S ribosomal RNA [26]. The inhibition of protein synthesis of the affected cells including endothelial cells leads to the apoptosis and eventual cell death. Platelets and coagulation factor deposition due to endothelial surface injury result in the formation of microthrombi and the TMA lesion which characterized HUS.

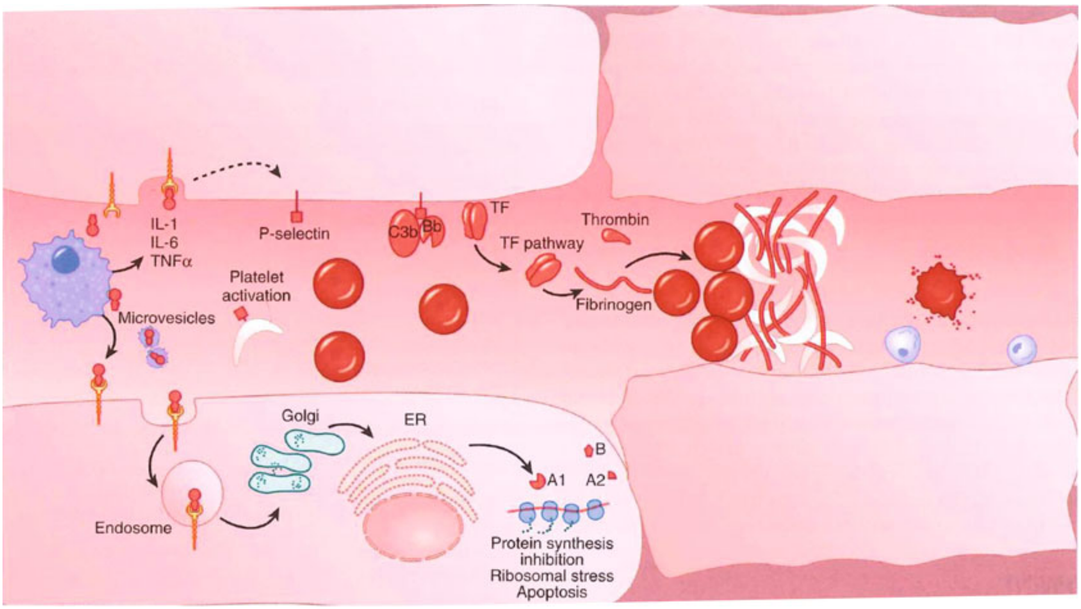


Fig. 3 Shema of Stx-mediated cell injury. In circulation, by binding subunit B of Stx to Gb3, Stx is internalized into the cell by endocytosis and transported in a retrograde manner to the endoplasmic reticulum. Thereafter, the subunit A translocates to the cytosol and interferes with ribosomal protein synthesis. The inhibition of protein synthesis of the affected cells leads to apoptosis and eventual cell death. Platelets and coagulation factor deposition due to endothelial surface injury result in the formation of microthombi and the TMA lesion. Reproduced from Kavanagh D, Sheerin N. Thrombotic microangiopathy. In: Yu ASL, Chertow GM, Luyckx VA, Marsden PA, Skorecki K, Taar MW, editors. Brenner & Recctor's the Kidney. 11th ed. 1178–95. Copyright 2020 Elsevier Masson SAS. All rights reserved

Stx-caused vascular injury stimulates thrombin release, resulting in increased fibrination, while increased plasminogen activator inhibitor-1 secretion by damaged vascular endothelial cells [27] inhibits fibrinolysis and promotes thrombosis. Exposure of Stx results in the rapid and persistent secretion of the unusually large von Willebrand factor (ULVWF) by vascular endothelial cells, thereby promoting the immediate adhesion of flowing platelets and the formation of large amounts of ULVWF-platelet strings. In turn, cleavage rates of plasma ULVWF-platelet strings by a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 (ADAMTS13) are decreased; thus, platelet thrombus formation is accelerated [28]. In addition, Stx induces the secretion of vasoconstrictor proteins by endothelial cells, such as endothelin-I [29], as well as various endothelial adhesion molecules and chemokines, which will promote leukocyte migration and adhesion [27]. Importantly, exposure to Stx increases the expression of Gb3 in targeted cells resulting in increased sensitivity to the toxin [30].

Involvement of Inflammatory Components

Although the pathogenesis of endothelial cell damage caused by inhibition of protein synthesis by Stx is necessary for the development of HUS, recent data has suggested that host factors, particularly inflammatory responses, are involved in the development and severity of HUS.

Polymorphonuclear Neutrophils

Increased peripheral blood leukocyte, particularly polymorphonuclear neutrophils (PMNs), was described as poor prognostic factors in HUS [31], suggesting a differential involvement of PMNs in HUS pathogenesis. Thus, it is believed that PMNs are directly involved in renal inflammation and endothelial cell damage via proteolytic enzymes released by degranulation and increased reactive oxygen species (ROS) production under the acute phase of HUS [32]. Oxidative stress is usually appropriately removed and balanced in vivo by endogenous antioxidants [33]. However, it has been reported the increase of thiobarbiturate reactive substances as indicators of lipid peroxidation [34], as well as higher levels of advanced oxidation protein products [35] and reduced superoxide dismutase activity [36], overall suggesting an oxidative imbalance in the acute phase of HUS.

Complement System

In some patients with STEC-HUS, low serum C3 levels [37] and increased factor Bb and soluble membrane attack complex (SC5b-9) [38] has been reported, suggesting at least transient activation of the alternative complement pathway. Additionally, it was shown in vitro that Stx binds to short consensus repeats 6–8 and 18–20 of complement factor H, impairing the alternative C3 convertase inhibition by factor H at the endothelial cell surface [12]. Therefore, Stx might directly activate complement alternative pathway in STEC-HUS.

Clinical Course (Fig. 4 [12])

The clinical manifestations of HUS are nonspecific, including fatigue, pallor, and decreased urine output. STEC-HUS frequently occurs after prodromal illness with abdominal symptoms due to STEC infection-associated gastroenteritis and enterocolitis. The symptoms of STEC infection-associated gastroenteritis such as abdominal cramps, diarrhea, nausea, and vomiting appear after an incubation period of 3–8 days (median 3–4 days) [39]. The predominant symptom is diarrhea, which usually starts as a non-bloody diarrhea, persists for 2–3 days, and progresses to bloody diarrhea in as many as 60–90% of STEC-infected cases [12, 40]. Abdominal pain is more severe in STEC infection-associated gastroenteritis than in other bacterial enterocolitis; additionally, defecation tends to be painful, but fever is rare [12, 40]. Moreover, STEC is rarely found to be invasive and detected in blood cultures. The

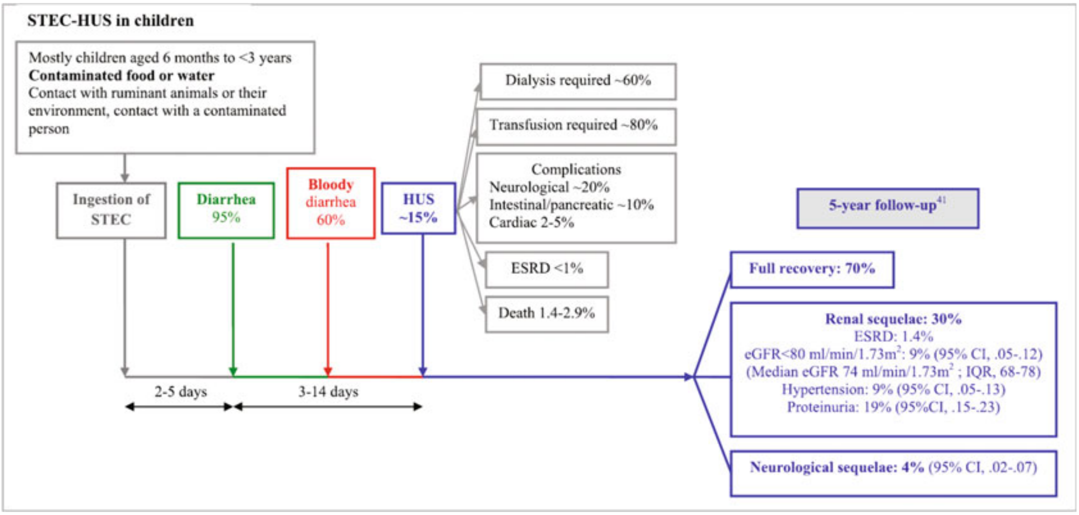

STEC-HUS in children
Mostly children aged 6 months to <3 years
Contaminated food or water
Contact with ruminant animals or their environment, contact with a contaminated person
Ingestion of STEC
Diarrhea 95%
Bloody diarrhea 60%
HUS ~15%
Dialysis required ~60%
Transfusion required ~80%
Complications
Neurological ~20%
Intestinal/pancreatic ~10%
Cardiac 2-5%
ESRD <1%
Death 1.4-2.9%
2-5 days
3-14 days
5-year follow-up[41]
Full recovery: 70%
Renal sequelae: 30%
ESRD: 1.4%
eGFR<80 ml/min/1.73m^2: 9% (95% CI, .05-.12)
(Median eGFR 74 ml/min/1.73m^2 ; IQR, 68-78)
Hypertension: 9% (95% CI, .05-.13)
Proteinuria: 19% (95%CI, .15-.23)
Neurological sequelae: 4% (95% CI, .02-.07)

Fig. 4 Clinical course and outcome of STEC-HUS in children. STEC: Shiga toxin-producing *Escherichia coli*; HUS, hemolytic uremic syndrome; ESRD, end-stage renal disease; eGFR, estimated glomerular filtration rate; CI, confidence interval. Reproduced from Bruyand M, Mariani-Kurkdjan P, Gouali M, Valk H, King LA, Hello SL, Bonacorsi S, Loirat C. Hemolytic uremic syndrome due to Shiga toxin-producing Escherichia coli infection. Med. Mal infect; 48:167–174. Copyright 2018 Elsevier Masson SAS. All rights reserved

colon can be quite severely affected, and the characteristic features of colon in patients with hemorrhagic colitis due to STEC infection are severe inflammation, primarily marked edema and easy bleeding, inflammation predominantly in the right side of the colon, and frequent appearance of longitudinal ulcer-like lesions as observed from the colonoscopic findings (Fig. 5) [41]. Abdominal computed tomography or ultrasonography [42] often shows swelling and thickening of the colon wall, particularly the right side of the colon [42] (Figs. 6 and 7). Diarrhea usually improves within approximately 1 week, and colon wall thickening improves gradually. Overall, 10–15% of patients with STEC infection develop HUS after a median interval of 7 days (interquartile range [IQR] 5–8 days) from the onset of diarrhea [31]. Once STEC infection has progressed to HUS, consumptive thrombocytopenia is observed as the first abnormality in most patients [40]. Hemolysis may result from mechanical fragmentation in passing through the turbulent blood flow in microvasculature due to partial occlusion by new platelet thrombi. Therefore, hemolytic anemia in STEC-HUS is Coombs negative, with the presence of schistocytes in the peripheral blood smear. The lactate dehydrogenase levels are elevated due to cell lysis, and haptoglobin levels are decreased due to the binding of free hemoglobin with haptoglobin and uptake by the reticuloendothelial system [25]. Although hemolysis in STEC-HUS generally precedes azotemia, some patients have shown elevated creatinine levels 1 or 2 days before the decrease in the packed cell volume [25, 40]. Oliguria and hematuria may result in anuria, depending on the

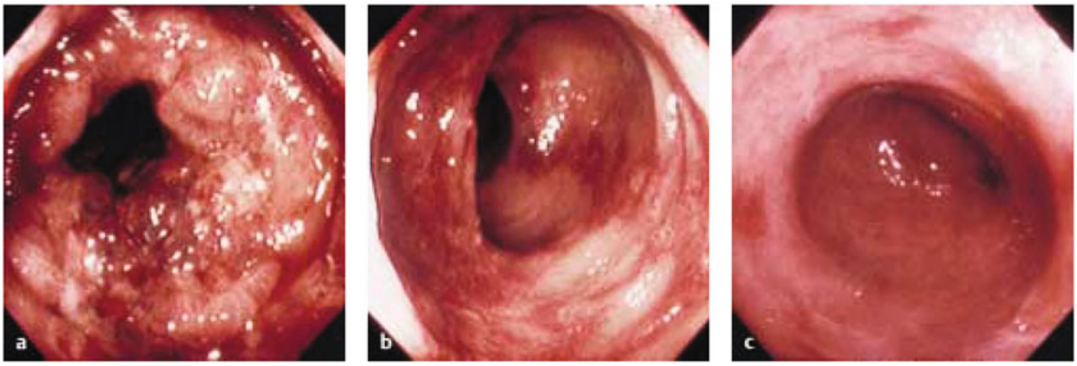
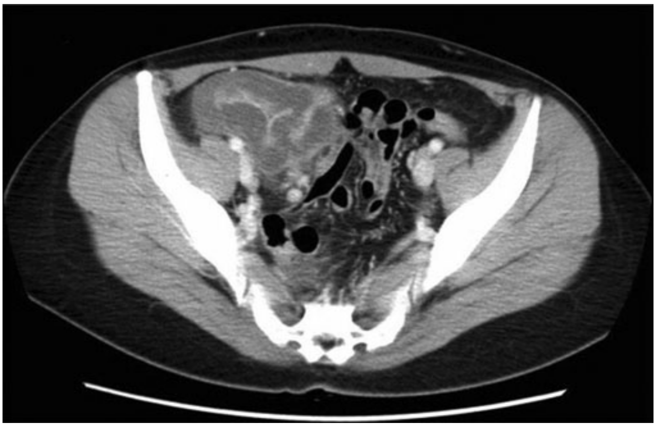


Fig. 5 Colonoscopic findings of hemorrhagic colitis due to O157 infection. (**a**) Findings in the ascending colon, (**b**) findings in descending colon, (**c**) findings in sigmoid colon. (Reprinted with permission from reference [41])

Fig. 6 Abdominal contrast CT images of STEC O157 infection. Edematous change of the right side of the colon from the cecum to the ascending colon. (Reprinted with permission from reference 42)



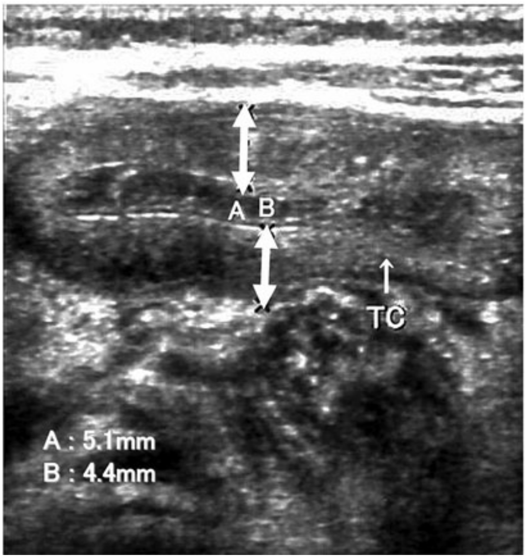


Fig. 7 Abdominal ultrasound images of hemorrhagic colitis due to STEC infection. Wall edematous thickening of the transverse colon (arrows). (A and B) diameter of the edematous thickening wall. (Reprinted with permission from reference 42)

disease severity. In the acute phase, approximately 50–60% of patients require renal replacement therapy (e.g., dialysis) [12, 31], and more than 90% of patients require transfusion including transfusion of red blood cells (RBC), platelet, and fresh frozen plasma [12, 31]. Although the kidneys and gastrointestinal tract are the most commonly affected organs in HUS, various organs including the central nervous system, pancreas, skeletal muscle, and myocardium are involved. Among the affected organs, involvement of the central nervous system is the most life-threatening complication in HUS, and it occurs in 10–25% of patients with STEC-HUS [12, 42–44]. According to a meta-analysis, diabetes due to pancreas involvement occurs in 3.2% of patients with HUS [45], and ischemic cardiomyopathy, life-threatening condition, may occur in 2–5% of patients with HUS [12]. The mortality rate, mainly attributed to neurological complications, has been reported to be 1–3% [31, 46].

Severe gastrointestinal involvement can result in severe complications such as hemorrhagic colitis, toxic megacolon, and transmural necrosis of the colon with gastrointestinal perforation or colonic stricture [47–51]. These complications occur in approximately 5–10% of patients [12, 47, 50], and the right side of the colon, including the transverse and ascending colon, is the most commonly affected bowel segment [47]. Histopathological examination of the colon revealed hemorrhage, edema, inflammatory cell infiltration, and necrosis [48], and necrotic lesions were observed in more than 80% of the examined cases [47]. In addition, abundant terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)-positive nuclei were observed in the tissue obtained from STEC-HUS patients compared with those of from controls [48]. These findings suggest abundant cell death due to apoptosis in the colon tissue of patients with STEC-HUS [48].

Pancreatic involvement in HUS more likely occurs in severe cases with central nervous system involvement or wherein acute dialysis is required [45]. A meta-analysis reported [45] that the incidence of diabetic complications in HUS in the acute phase was estimated to be 3.2% in the pooled incidence. Of 44 patients whose long-term outcomes were reported, 23% died in the acute phase, and 38% of the surviving patients remained diabetic, requiring insulin administration. Concerning the pathogenesis of insulin deficiency resulting in diabetes in HUS, the mechanism may be directly due to HUS with TMA of the pancreatic microvasculature [45], according to autopsy findings that TMA lesions were restricted to the islets of Langerhans with or without slight necrosis of the adjacent tissues [52].

Central nervous system involvement is common in HUS, and its symptoms including lethargy, irritability, seizures, cortical blindness, paresis, and coma have been reported in 10–25% of the affected children [12, 42–44]. Various complications associated with renal insufficiency, including hypertension, electrolyte abnormalities, and uremia, may contribute to the development of neurological manifestations [43]. Although the mortality rate in patients with HUS has decreased to less than 5% due to neurological symptoms, it has been reported that 54% [31] and 71% [46] of deaths were due to neurological involvement and that 17.3% of cases with severe neurological symptoms including coma and convulsion in the acute phase died and 23.1% had severe disabilities [53]. Therefore, it is still necessary to pay attention to the presence and severity of neurological manifestations complicated with HUS. In morphological analysis, the autopsy findings of the brain of 32 patients with HUS revealed random distribution of TMA throughout the brain in 11 cases [52]; the analytical findings of head magnetic resonance imaging (MRI) in HUS patients with neurological symptoms showed that all the structures of the central nervous system had the lesions [53]. However, no correlation was reported between the location of MRI abnormalities and the final prognosis [53].

For renal involvement, oliguria and hematuria may result in anuria, depending on disease severity. In the acute phase, approximately 50–60% of patients require renal replacement therapy (e.g., dialysis) [12, 31]. The mean duration of dialysis was 10 days (IQR, 6–15 days) [44]. Multivariate logistic regression analysis revealed that the longer the duration of dialysis, the higher the risk of presenting sequelae at 1-year, 2-year, and 3-year monitoring and that the median duration of dialysis was 15 days in children with long-term problems. The duration of dialysis required for children with long-term problems was longer than that in those without long-term problems (median duration: 9 days). These findings may suggest that the required dialysis period is a disease severity marker.

Long-Term Complications (Prognosis)

Owing to reduction in case fatality and improvement in the prognosis, chronic complications are more apparent in long-term survivors. It is reported that 1.4% of patients with HUS develop to end-stage renal disease [46], and 38% of survivors who were complicated with insulin-dependent diabetes mellitus required long-term insulin administration [45]. Additionally, 5 years after HUS diagnosis, approximately 70% of the patients fully recovered. However, the remaining 30% of patients showed neurological sequelae, persistent hypertension, or impaired renal function. Hypertension and proteinuria developed in 18% of patients who had no sequelae 1 year after the acute phase [46]. Therefore, long-term follow-up and care based on development of CKD is essential [46, 54].

Acute Management of HUS

STEC-associated HUS despite its relative rarity is still a major factor of morbidity for children and remains one of the most common reason of acute intrarenal kidney injury that requires dialysis in childhood [55].

Unfortunately, despite ongoing effort, there is still no specific cure or intervention for STEC-HUS. Therefore, foundation of therapeutic approach is the best symptomatic care that allows good glomerular perfusion and prevents long-standing damage to kidneys and other organs by providing adequate hydration, blood pressure control, and elimination of effects of nephrotoxic insults [56].

General Management of Patient with STEC-HUS

General aspect of treatment are symptomatic, following development and severity of the disease progression and its specific complications. Considering how rapidly the disease can progress from colitis to systemic manifestation of HUS with AKI, we would recommend that patients with developing STEC-HUS are admitted to a pediatric nephrology center with capacity for rapid initiation of renal replacement therapy and careful monitoring and backup of high dependency unit when severe central nervous symptoms develop.

The care follows patient symptoms, in the first instance development of acute kidney injury of variable degree. The general aspects of care for patients with AKI in terms of electrolytes management, fluids, and initiation of RRT should be followed; therefore, we recommend to follow appropriate recommendations in chapters on this topic. Patients usually require renal replacement therapy where both modalities – peritoneal and hemodialysis – are equal in terms of efficacy and risks. Continuous renal replacement therapy is also utilized, particularly in patients with severe presentation or complications or when cardiovascularly unstable. There is no specific proof that early RRT improves outcome.

Antihypertensive therapy might be necessary and could potentially reduce secondary cerebral complications like PRES. The usual recommendation is first-line treatment with calcium channel blockers. ACE inhibitors do not show specific advantage, and their use should be based on specific needs and risk of renal hypoperfusion.

Pain management avoiding NSAIDS and adequate nutrition support should be a routine part of care. Interestingly, fluid management showed some alleviation of abdominal pain. It is speculated that improved perfusion plays some role in it.

Antimotility agents are discouraged in STEC-HUS as they may prolong possibility of toxin exposure. On the opposite, bowel lavages or drugs speeding up the bowel content passage were suggested to decrease toxin exposure.

Fluid Management

In hemorrhagic enterocolitis due to STEC infection, dehydration often results from frequent diarrhea, severe abdominal pain, nausea, and decreased fluid intake due to vomiting. Recent studies have demonstrated that hemoconcentration and dehydration, which often coexist at HUS onset, are risk factors for more severe disease conditions including renal involvement [57, 58]. In addition, it has been reported that appropriate volume expansion using isotonic solution with careful monitoring of the patient's hydration state may be able to reduce or prevent severe HUS symptoms [59, 60]. A systematic review demonstrated that clinically dehydrated patients had an OR of death of 3.71 (95% CI, 1.25–11.03; $I^2 = 0\%$) and that intravenous fluid administration up to the day of HUS diagnosis was associated with a decreased risk of renal replacement therapy (OR 0.26 [95% CI, 0.11–0.60]) [61].

Hematological Symptoms Management and Treatment with Blood Products

Recommendation of blood products infusion is based on clinical need and is usually a necessary part of care in STEC-HUS patients. Packed RBC are given based on clinical grounds and symptoms of anemia like tachycardia and tachypnea. The cutoffs for transfusion is debated. ECUSTEC trial (https://www.birmingham.ac.uk/research/bctu/trials/renal/ECUSTEC/index.aspx) uses cutoff for hemoglobin of 70 g/L; however, some recommendations are based on symptoms of anemia or have much lower cutoffs and more guided by symptoms severity.

Thrombocytopenia is a defining hallmark of HUS and is seen as a complication for interventions, particularly need of placing a dialysis intravenous line or PD catheter. Platelet transfusion is used to reduce risk of bleeding; however, retrospective study has not showed increased risk regardless platelet infusion or lack of it [62]. Pathology of the disease call for caution in giving additional platelets as this can further fuel the process of microthrombosis. Retrospective study has not showed significant impact of platelet transfusion on HUS severity [63]; therefore, the platelet infusion should be weighted based on individual patient risks of bleeding.

Plasma infusion, plasma exchange, and immunoadsorption have showed some effect on neurological symptoms in single case reports; however, this was not demonstrated on larger samples [64]. Therefore, plasma therapy has not been found beneficial in children with STEC-HUS and could create even increased risk, particularly in smaller age groups [55].

Antibiotics in STEC-HUS

Antibiotic therapy is a matter of controversy and generally not recommended. In vitro studies show antibiotics lead to increased production and release of Stx, and prospective study and retrospective meta-analysis on more than 1000 children showed increased incidence in children receiving antibiotic. There was also no benefit seen in reducing other symptoms of STEC infection [65]. On the other hand, recent report separating different antibiotics showed detrimental effect of beta-lactams and trimethoprim/sulfamethoxazole and some benefit of fosfomycin and fluoroquinolones [66].

Special consideration is required in cases where intestinal gangrene develops, bacterial superinfection is suspected, or septic features are present. These situations need to be weighed on individual risk benefit for the patient.

Preventive Measures to Decrease Chance of Developing STEC-HUS and Additional Treatments and Therapies in Development

Beyond the currently recommended symptomatic approach, there is an ongoing effort to find a treatment or approach that will diminish the mortality and morbidity.

The disease still creates significant burden and remains without targeted treatment and preventive strategy except epidemiological interventions and infection prevention.

This unmet need creates an opportunity for developing strategies of reservoir elimination or immunization, prevention of toxin exposure, and most importantly for therapies that will stop and prevent further damage in already established endothelial injury. Despite numerous concepts summarized in recent review [67] (Table 3), there are very few of them that reached the level of clinical intervention or trial.

The epidemiological prevention and reservoir surveillance still has its place. Early identification and microbiological diagnosis of STEC can reduce and curb outbreaks when sources are identified rapidly [18].

Strategies to reduce reservoir infection by vaccination or specific diet given to cattle are in development but have not been largely implemented or tested, particularly due to rarity of complications and need of large-scale intervention in cattle and wild ruminants reservoir.

The second line in the concepts of preventing HUS lies in reducing toxin transfer in already infected individual from the gut to the bloodstream. Most advanced was a trial of Synsorb PK, which ought to bind toxin in the gut. Unfortunately, the trial has not showed benefit of the drug in children [68]. Similar conceived are trials where monoclonal antibodies or peptides should prevent reaching STx in the bloodstream to the areas with GB3 receptor [69]. These shown some promise in animal models; however, their practical usage seems limited due to very short window between infection and identification of the patients in risk of developing HUS and that our knowledge of precise location and activity of toxin in the pathogenesis is still sparse [67].

The final option of preventing damage of the STEC infection is to reduce final steps like inflammation and coagulation. Steroid treatment, IVIG, and anticoagulation and antiaggregating treatments were used in small studies, where they had not shown benefit [55].

Several reports indicated that in patients with STEC, alternative pathway of complement is activated, and levels of consumption in some report correlate with severity [70]. Eculizumab is a humanized anti-C5 antibody that blocks terminal pathway of complement and showed remarkable success in genetic complement-driven cases of atypical HUS [71]. Eculizumab showed promise to decrease extrarenal complications of STEC-HUS which was reported in seminal case series of three children [72]. However, large opened trial during the 2011 O104:H4 outbreak in Germany has not showed benefit of the drug in adults [64]. Several collated case series remain equivocal [73]. The conundrum might be resolved by two placebo-controlled randomized trials ECUSTEC (UK) and ECULISHU (NCT02205541 France) that are still ongoing and should report their results soon [74].

Streptococcus pneumoniae-Associated HUS

Non-STEC-HUS can be caused by other invasive infections such as the Gram-positive, encapsulated bacterium *Streptococcus pneumoniae* (*S. pneumoniae*). Primarily, it causes infections of the upper (sinusitis) airways but also otitis media and later lower airways (pneumonia). Its invasive and systemic form, IPD, causes multiorgan damage, like pulmonary empyema and meningitis. Some patients with IPD also develop HUS (P-HUS). Although P-HUS represents a low but significant number of all HUS cases, robust and definite data about pathophysiology, clinical course, treatment, and outcome are still lacking, which could partly be due to the fact that it is underdiagnosed in patients with IPD. During the

Table 3 Summary of developed interventions based on mechanisms (first line of table) for HUS treatment. Summarized from review Muhlen et al. 2020

Shiga toxin binding	Scavenging Shiga toxin mimicking GB3 receptor	Intracellular interference with Stx	Inhibitors	Natural products	Inflammation/ immunomudualtion	Antibiotics
Oral bovine colostrum[a]	Synsorb Pk[b]	Chloroquine[c]	Pyocins[c]	White carob tree[a]	Eculizumab[a,b]	
Recombinant antibodies in plants for cattle feed (elimination from reservoir)[c]	Starfish/Daisy carbohydrate ligands, Super Twig (carbosilan)[c]	Retro1,2 Ac-PPP-tet, TVP[c]	Phages[c]	Ziziphus mistol extract[c]	Steroids[a]	
Humanized Stx targeting antibodies like Urtoxazumab[c], 13C4,11E10 Stx targeting camelid antibodies[c]	Neutralizing peptides binding to GB3[c]			Elagitannin[c]	IVIG[a]	
	Oral administered bacteria expressing GB3[c]					
	Nanoparticles displaying Stx ligands[c]					
	GB3 expression inhibitors[c]					

[a] Human cases/series
[b] RCT
[c] Animal study/in vitro

last years, it has become evident that P-HUS is caused by several factors and mechanisms.

Pathophysiology

A current concept is that in IPD, members of the bacterial neuraminidase enzyme family (NanA, NanB, NanC) lead to, by cleaving sialic acid (*N-acetylneuraminic acid*) from glycoproteins to the exposure of the normally hidden Thomsen-Friedenreich Antigen (T-Antigen, Galß1-3GalNac) on the surface of various cells, like erythrocytes or renal endothelial cells [75, 76]. The presence of one (*NanC*) as a predictor for the development of P-HUS as being additive to the presence of *NanA* and *NanB* has been proposed but has not been confirmed entirely [77]. As a condicio sine qua non, circulating, i.e., already existing or newly generated, antibodies of the IgM-Type detect and bind to the T antigen (TA) [78]. As a consequence, downstream immune mechanisms then damage or destroy T antigen-expressing cells (RBC, endothelial cells), leading finally to TMA and MAHA. Initially anticipated as being specific and sensitive for IPD-associated HUS, only 60% of all P-HUS and IPD patients are encountered with a positive Coombs Test [79–81]. Chen et al. described that 66% of patients with IPD were found to have TA activation, but TA activation was significantly correlated with HUS and a more severe clinical course [82]. On the other hand, only half of the TA positive patients did develop HUS. By using isolates of P-HUS patients, Meinel et al. found that pneumococci bind human plasminogen, mediated by the bacterial surface proteins Tuf (Elongation factor Tu) and PspC (pneumococcal surface protein C) [83]. When activated to plasmin at the bacterial surface, the active protease degraded fibrinogen and cleaved the complement factor C3b. Likewise, PspC, might act as pneumococcal plasminogen receptor. Plasmin generated on the surface of pneumococci, in turn, damages endothelial cells, exposes the underlying matrix, and engages complement activation.

Galectin-3 (Gal-3), an endogenous T antigen binding protein that links in high concentrations a supramolecular structure of cell surfaces and glycoconjugates, was found significantly higher in IPD patients when compared to normal healthy controls and was even higher in patients with IPD and P-HUS. As such, this mechanism could in turn lead to complement activation [84, 85].

In some of these studies, complement activation has been identified to play a role in the pathophysiology of IPD and P-HUS. Therefore, studies have focused directly onto the role of the complement system in pathogenesis and also treatment of P-HUS. It has been reported that complement activation via the alternative pathway can be triggered by desialylation on RBC [86–88]. In turn, the binding capacity complement factor H to C3 convertase, and this lack of inhibition leads to complement activation and endothelial damage [89]. Studies have shown that complement factor C3, in fact the only parameter that is readily available in the majority of hospitals, is decreased in several patients with IPD and HUS [90, 91].

Besides a transient dysregulation of the complement pathway, studies have also identified genetic factors that contribute to permanent activation, or states where, like in patients with aHUS, external factors trigger overt disease, like infections, malignant hypertension, or pregnancy. In clinical studies, variations and mutations in the complement pathway of some patients with IPD and P-HUS have been detected [92]. As more patients have been reported with variations in the complement system, it has been investigated if patients might benefit from the treatment with the C5 blocker, eculizumab.

Clinical Course

Pneumococcal vaccine coverage has increased by including more and more subtypes. In several countries, different vaccinations are available, like PPSV23, a polysaccharide-based vaccine which is restricted to patients of the age 2 years and older; the PCV10, a conjugation-based vaccine restricted to the age of 5; and PCV12, a conjugation-based vaccine with no age restriction. The latter includes also the serotype 19A, that has been reported as the most prevalent serotype in P-HUS (54.5%) in 2010 [80]. However, P-HUS is still most prevalent in younger children and mostly in those below the age of 2. A few days after the onset of fever and other IPD signs and symptoms, P-HUS manifests, in most of the cases between 3 and 13 days [93]. About 60% present with pneumonia-associated empyema (Fig. 8) and meningitis, which contributes per se to the high morbidity and mortality in this cohort and, certainly, to the rather poor outcome when compared to STEC-HUS [94]. P-HUS patients have, compared to STEC-HUS patients, more complicated clinical course. They were reported to have longer periods of oliguria and anuria, therefore a longer need for renal replacement therapies and need more days of intensive care [80, 94, 95].

Management

Although several molecular mechanisms of P-HUS have been identified, supportive management and targeted antimicrobial therapy represent still the major strategy as it is in all patients with infection-associated HUS. IPD-associated complications like lung empyema, effusions, and pneumothorax and the difficult airway management are often the limiting problems of treatment. Due to the concept of preformed IgM antibodies to the T antigen, washed RBC are still recommended. The beneficial use of IVIG by the

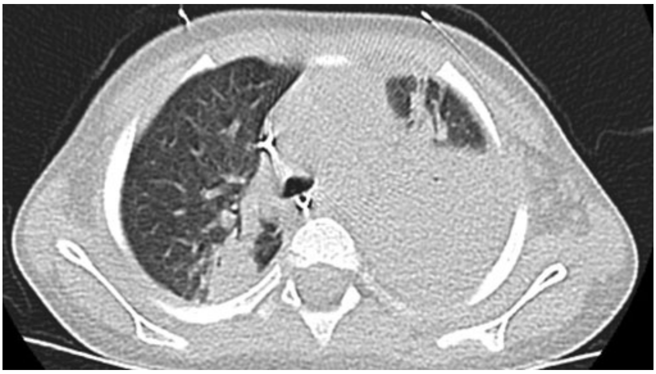


Fig. 8 An 18-month-old patient with P-HUS. The CT scan reveals heavy pulmonary involvement with effusion and empyema

concept to bind circulating neuraminidase has been reported [90]. The decision whether peritoneal or hemodialysis should be introduced is dependent on the patient's individual situation (availability of vascular access, bleeding complications, etc.). Especially, in the clinical situation of IPD and disseminated intravascular coagulation (DIC) and multiorgan failure, the coexistence of P-HUS should be vigorously searched for. In the presence of IPD and AKI, renal biopsy is often not helpful, since MAHA and TMA can be caused by other factors than P-HUS. As there is increasing evidence of transient or permanent dysregulation of the complement system in P-HUS, attention should be paid in the early introduction of C5 Blockers. Before initiation of complement inhibition (eculizumab or ravulizumab), complete diagnostics of complement (except genotyping) should be obtained. If the patient is not completely immunized against encapsulated bacteria (pneumococci, meningococci and *Haemophilus influenzae*), sufficient antibiotic coverage must be ensured. Moreover, other situations that could present with TMA and MAHA should be excluded, as other therapies are available (i.e., caplacizumab for TTP) [96].

HUS Associated with *Shigella dysenteriae* Type 1, Influenza Virus, and Other Infections

HUS Associated with *Shigella dysenteriae* Type 1

Shigella is a non-motile Gram-negative facultative anaerobic rod, belonging to the family *Enterobacteriaceae* [97]. In 1897, the microbiologist Kiyoshi Shiga identified this bacterium as the causative agent for dysentery during an epidemic in Japan, which was associated with high mortality [97]. Although mortality due to *Shigella*-associated diarrhea has declined substantially during the past three decades due to increased access to antibiotics and oral rehydration therapy, *Shigella* was the second leading cause of diarrhea-associated mortality in 2016 among all ages accounting for about 13.2% of all diarrhea-associated death [98]. In the 2012 EU/EEA surveillance report, the notification rate for *Shigella* was 1.8 cases/100,000 persons [9], and in 2016 the incidence of *Shigella* in the United States was reportedly 3.92 cases/100,000 person [99]. *Shigella* is an antigenetically diverse pathogen that comprises four species including *Shigella flexneri, Shigella sonnei, Shigella dysenteriae, and Shigella boydii*. *S. flexneri* is the leading cause of endemic shigellosis in low-income and middle-income countries. Further, *S. sonnei* has been reported as the second most common *Shigella* species in low-income and middle-income countries and the leading species in high-income countries. *S. dysenteriae* has reportedly almost disappeared since the late 1990s, with the United States reporting only 18 cases in 2016 (0.1%, <0.01/100,000 persons/year) and the EU/EEA reporting 132 cases in 2012 (2.3%).

Since HUS associated with *Shigella dysenteriae* was first recognized in Bangladesh in 1975 [100], nearly 500 cases of HUS in shigellosis outbreaks have been reported as of 2012 [101]. Although a small number of adult cases have been reported, most instances of HUS secondary to shigellosis are pediatric cases [101]. Despite relatively few case numbers, the literature has elucidated both diagnostic considerations for HUS and the likely clinical findings through the disease progression.

Manifestations of HUS develop late in the clinical course of shigellosis and may occur more than a week after onset of diarrhea. There are clinical reports that describe onset of bloody diarrhea due to shigellosis 4 to 17 days prior to the onset of HUS [101]. Diagnostically, leukocytosis and hyponatremia have been observed as likely preceding findings or associated signs of HUS other than the typically observed triad. According to Butler in summarizing seven reports, the incidence of HUS secondary to shigellosis ranged from 6–45%, with an overall incidence of 13% and a post-HUS mortality of 36% [101]. The incidence and mortality rate of HUS associated with *Shigella dysenteriae* type I are higher than those of STEC-HUS. *Shigella dysenteriae* type 1 has enteroinvasive properties that STEC does not and thus presents a higher risk for bacteremia, requiring a special focus on early treatment.

Appropriate antimicrobial treatment for HUS associated with *Shigella dysenteriae* type 1 during the early stages of infection, based on the specific drug sensitivity, is necessary [102]. It was reported that of 378 confirmed *Shigella dysenteriae* infection in which appropriate antimicrobial treatment started within 96 h, only one child developed HUS [103]. Furthermore, in 20 children with *Shigella dysenteriae* type 1 infection, fecal Stx was chronologically quantified after the onset of antibiotic therapy. In many cases, fecal Stx levels serially decreased and became undetectable at 72–120 h, and none of the 20 children developed HUS [103]. These results clearly support the recommendation for early treatment with appropriate antibiotics for *Shigella dysenteriae* infection.

Influenza Associated HUS and HUS Associated with Other Viral Infections

Influenza-associated HUS was first reported in 1971 [104], and since then, only 25 cases have been accumulated in the literature up until 2018 [105]. It is mostly associated with influenza A virus, specifically A (H3N2) and A (H1N1), with few reported cases associated with influenza B. In the literature survey [105], all 25 patients manifested the typical triad of HUS, 10 (40%) received renal replacement therapy, and 10 (40%) had central nervous system involvement. Of the seven patients who received it, all Coombs tests were negative. The prognosis was generally favorable with only three deaths (14%), and all but one surviving patient reported recovery of their renal function [105]. Although there is an established association between influenza virus infection and HUS, the underlying mechanisms remain speculative. The influenza virus has neuraminidase productivity like *Streptococcus pneumoniae*. However, in vivo neuraminidase released by influenza virus is expressed on the viral membrane at a lower quantity than *Streptococcus pneumoniae*, and its contribution to the pathogenesis of HUS has not yet been demonstrated. In addition, other factors such as direct injury and activation of vascular endothelial cells, platelet activation [106], and systemic complement activation [107] by influenza virus may be involved in the pathogenesis. In the literature survey [105], 8 of 25 patients underwent genetic screening, and the mutations in the gene for alternative pathway of complement were identified in 7 of the 8 patients, suggesting that influenza infection is a trigger for the development of HUS in patients with alternative pathway of complement regulator protein haploinsufficiency. Therefore, to clarify the pathogenesis of influenza-associated HUS, it is necessary to confirm the diagnosis of influenza virus and the disease type of TMA using measurements of ADAMTS13 activity, the identification of pneumococcus infection, the Coombs test, and genetic test for complement regulator protein.

It is reported that HUS has also been associated with HIV, cytomegalovirus, HHV6, parvovirus B19, and others [2].

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Acute Post-Streptococcal Glomerulonephritis

Hong Xu and Michael J. G. Somers

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Abstract

Acute post-streptococcal glomerulonephritis is the prototype for a postinfectious immune-complex mediated glomerulonephritis. Clinical manifestations follow a latent period of 1–6 weeks after a streptococcal skin or upper respiratory tract infection. Epidemic infections have been reported. Disease prevalence tends to be highest in economically disadvantaged geographic areas and populations. Disease pathogenesis has highlighted certain cell surface M virulence proteins and several putative nephritogenic antigens have been proposed, with activation of the complement pathway playing a key role. The disease phenotype is broad, ranging from asymptomatic microhematuria to a rapidly progressive glomerulonephritis (RPGN). Peak prevalence is in young school-aged children, with a preponderance of boys affected. Gross hematuria and hypertension are the most common clinical characteristics, with a third or more of affected children with oliguria or azotemia. Nephrotic syndrome is less common than a nephritic presentation. Depression of C3 complement levels and positive anti-streptolysin O and anti-DNAse B titers help to confirm the diagnosis. Kidney

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biopsy is infrequently necessary, but typically reveals a proliferative and exudative glomerulonephritis on light microscopy, with C3 and IgG deposition on immunofluorescence, and subepithelial electron-dense humps as well as subendothelial deposits. Treatment is generally supportive, with focus on volume overload and hypertension, with most children recovering rapidly without long-term kidney residuals. Significant acute kidney injury requiring dialysis is usually associated with an RPGN with crescentic changes that is most commonly treated with pulse intravenous steroids, though there is no evidence-based support for immunomodulatory therapy.

Keywords

Post-streptococcal · Glomerulonephritis · Postinfectious · Immune complex · Nephritic syndrome · Hypocomplementemia · Anti-streptolysin O

Introduction

Acute post-streptococcal glomerulonephritis (APSGN) is the prototypical postinfectious immune-mediated glomerulonephritis. It is associated with a previous skin or throat infection, most often caused by group A streptococcus (*Streptococcus pyogenes*) and occasionally by streptococcus group C or G. APSGN is the most common form of glomerulonephritis in children, and like other diseases caused by group A streptococcus, it is seen disproportionately in economically disadvantaged populations [5].

APSGN is seen classically between 2 years of age and late adolescence, with primary school-aged children more frequently affected. Boys are afflicted twice as commonly as girls. The latent period between infection onset and nephritis has often been reported as shorter with respiratory illness (7–10 days) than skin infection (2–6 weeks), though there is little published clinical evidence that latent periods vary this broadly. Seasonally, respiratory illness-associated ASPGN predominates in the winter and spring whereas skin infection-associated ASPGN is seen more commonly in the summer and autumn. The typical clinical presentation for ASPGN is a classic nephritic syndrome characterized by hematuria, edema, hypertension, and oliguria.

The prevalence of APSGN varies widely and is influenced by geographic, socioeconomic, host, and bacterial factors. The incidence of APSGN has decreased worldwide, particularly in more economically advantaged countries. In developing countries, the annual burden of APSGN remains at a level of least 9 cases per 100,000 inhabitants [61].

Epidemiology

APSGN may occur in case clusters related to streptococcal epidemics or in isolated patients. Epidemic outbreaks as a consequence of both upper respiratory and skin streptococcal infections have occurred worldwide, with notable incidents in the Red Lake Reservation in Minnesota [2], in Port of Spain, Trinidad [47], in Maracaibo, Venezuela [54], and in the Northern Territory of Australia [31]. A recent epidemiological study of children with newly diagnosed nephritis reported that the children of indigenous people in Central Australia have the highest APSGN incidence, with nearly 40% diagnosed with a preceding streptococcal infection, predominately from a skin infection within the preceding month [18].

In recent decades, the reported incidence of APSGN has been declining, probably related to easier and earlier access to appropriate medical care for streptococcal infections in more areas of the world. In developed countries, there have also been reports of the overall severity of APSGN decreasing [24], while in certain high-risk indigenous population such as in French Polynesia, massive proteinuria, congestive heart failure, and kidney failure are still frequent features [9]. In children with a glomerulonephritis who undergo kidney biopsy, histology manifesting endocapillary proliferation such as expected with APSGN is the most common finding in children from economically disadvantaged areas and in areas with a high proportion of indigenous people [16, 31, 56].

The striking difference in APSGN incidence as a function of access to resources is underscored by the findings in one meta-analysis of 11 population studies that showed an ASPGN rate as high as 24.3 per 100,000 in less developed countries and as low as 2.0 per 100,000 in more developed regions. This study also estimated that over 470,000 cases of APSGN occur annually, with 97% in less developed countries [14].

Pathogenesis

APSGN is an immune complex-mediated disease that is not just due to a single streptococcal antigen and its antibody. Rather, kidney damage may be the result of different factors acting at the same time related to both streptococcus and host factors [35]. Several mechanisms may participate in the pathogenesis of kidney damage (Table 1).

Nephritogenic Antigens Traditionally, APSGN was considered to be caused by a nephritogenic antigen present in group A streptococci. *Streptococcus pyogenes* of M types 1, 2, 4, and 12 were associated with epidemic nephritis following upper respiratory infections, and M types 47, 49, and 55 were associated with epidemic nephritis following pyoderma. Nephritis may also follow infections with non-group A streptococcus as evidenced by an epidemic of group C streptococci in Brazil [7] and several similar clusters of cases [38].

Throughout the years, many putative streptococcal nephritogenic antigens have been studied without definitive confirmation of any causal relationship with glomerulonephritis [55]. The nephritogenic potential of streptokinase was investigated by in mice using the experimental tissue cage model, and demonstrated glomerular streptokinase deposits in association with hypercellularity, complement deposition, and proteinuria [39]. Subsequent studies by this group evaluated allelic variants of streptokinase using site-specific integration plasmids, and this work demonstrated the nephritogenic properties of the nephritis-associated streptokinase gene *ska I [40]*.

Presently, there is particular focus on two streptococcal antigenic fractions as to their nephritogenicity, the nephritis associated plasmin receptor (NAPlr) identified as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [42, 72], and the streptococcal pyrogenic exotoxin

Table 1 Pathogenetic mechanisms participating in acute post-streptococcal glomerulonephritis

Mechanism	Evidence	Reference
Nephritogenic antigens (NAPlr, SPEB, streptokinase, others)	NAPlr and SEPB demonstrated in renal biopsies	[40, 42, 48]
Circulating immune complexes	Circulating anti-SPEB and anti-NAPlr antibodiesin APSGN patients	[46, 72]
In situ Immune complexes (cationic antigens)	SPEB co-localized with complement in glomeruli and demonstrated in the subepithelial electron-dense deposits (“humps”) in APSGN	[7]
Autoimmunity (anti-IgG, other)	Neuraminidase is produced by some nephritogenic streptococci. Serum neuraminidase activity in APSGN patients	[4, 54, 59]
Anti-Ig (induced by the loss of sialic acid of the IgG or binding of the Fc fragment of IgG to type II receptors on the surface of group A streptococcus)	Serum anti-IgG titers; Renal anti-IgG deposits	[13, 46, 54, 58]
Other autoimmune reactivity	Anti-DNA, anti.C1q, ANCA and anti-cardiolipin antibody demonstrated in serum	[3, 20, 29]
Other		
Increased plasmin activity in glomeruli (facilitating immune complex deposition	Co-localization of plasmin and NAPlr in glomeruli. Increasedurinary plasmin activity	[41, 42, 72]
Neuraminidase-induced glomerular infiltration of desialised leukocytes	Desialised leukocytes accumulate in the glomeruli of patients with APSGN	[30, 34]

(erythrotoxin) B (SPEB) and its zymogen precursor (zSPEB).

Work by Fujino et al. [19] has shown that the expression and gene sequence of NAPlr is present in streptococci from several groups. Anti-NAPlr antibody levels are found in 92% of the sera from convalescent APSGN patients and in 60% of the uncomplicated streptococcal infections in Japan [72]. NAPlr is present in early biopsies of APSGN, and since it is not co-localized with complement or IgG, its role as a nephritogen is thought to be related to its plasmin-binding capacity which facilitates immune complex deposition and inflammation [42].

The nephritogenic potential of plasmin-binding activity has been noted not only in NALPr but also in SPEB, streptokinase, and enolase. Some feel that this property may represent a common nephritogenic property of both NALPr and SPEB, since both of these antigens were identified by their group in the mesangium of biopsies of APSGN [41, 57, 72]. Along with this possibility, patients with APSGN exhibit an increase in urinary plasmin-like activity [41, 42].

SPEB, an extracellular cysteine proteinase, is a cationic antigen that is co-localized in the glomeruli with complement and Ig deposits. SPEB is the only streptococcal antigen that has been demonstrated within the electron dense subepithelial deposits (humps) that are the hallmark of APSGN [8]. Many studies have postulated that this localization is facilitated by the cationic charge of this antigen [69]. High anti-SPEB/zSPEB antibody titers have been found in the vast majority of convalescent sera sampled from APSGN patients in Latin America [46]. SPEB/zSPEB induces chemotaxis and increases angiotensin II production by mesangial cells [68]. Recent studies have demonstrated that the Fc portion of antibodies directed to SPEB binds to the C-terminal domain (rSPEB 345-398), and immunization with this domain prevents group A streptococcal infection in mice [65].

The attractiveness of a charge-related GBM injury in the pathogenesis of APSGN has led Zhang et al. [74] to examine if histones that are cationic elements may be part of the pathogenesis of APSGN. These studies showed that histones enter the circulation after streptococcal lysis and are capable of inducing in situ immune-complex formation.

Finally, recent evidence suggests that there is not a single nephritogenic antigen, since studies have shown that the gene that encodes for SPEB was absent in the *Streptococcus zooepidemicus* isolated from patients in a Brazilian epidemic [10]. Notably, in studies of this *Streptococcus zooepidemicus* strain, 33 of 44 APSGN patients were seropositive for the Szp5058 fusion protein that was isolated from the causative streptococcal strain [38], leading to speculation that the anti-phagocytic properties of Szp proteins may play a role in the pathogenesis of glomerulonephritis. Both NALPr and SPEB are capable of inducing monocyte chemoattractant protein 1 (MCP1) and IL-6 in mesangial cells [15, 27] and SPEB elicits the release of a variety of cytokines and interleukins from peripheral blood leucocytes [67].

Humoral and Cellular Immunity Activation of the complement system is a consequence of the glomerular antigen/antibody reactivity. The alternative complement pathway is usually most active in APSGN, with depression of C3 complement levels, although some may also have a reduction in C1 and C4 levels. In these patients, Protein H, a surface streptococcal protein, in combination with IgG may activate the classical complement cascade [11]. In addition, in some patients there may also be complement activation by the lectin pathway [43], but APSGN may also develop in individuals who are genetically unable to activate this pathway [62].

In addition to humoral immune initiation, cellular immune mechanisms are also activated in APSGN. It has long been known that there is cellular adhesion molecule (ICAM-1, LFA-1) overexpression with infiltration of lymphocyte and macrophages in the glomeruli of these patients [44, 45]. Higher numbers of CD4-positive lymphocytes are present in renal biopsies obtained in the first 3–4 weeks of disease and decrease in frequency afterwards [44]. Increased glomerular expression of IL-8 has been found to correlate with neutrophil infiltration and transforming growth factor–β expression with mesangial expansion [32].

Autoimmune Reactivity A number of autoimmune findings have been reported in APSGN, most notably anti-IgG reactivity. In the first week of disease, rheumatoid factor cryoglobulins are present in about two-thirds of patients. Furthermore, anti-IgG glomerular deposits are frequently found on biopsies, and anti-IgG reactivity has been documented in the IgG eluted from the kidney in a fatal case of the disease ([54]; [55]).

The anti-IgG reactivity may be the result of autoantigenic modification mediated by Ig desialization caused by streptococcal neuraminidase. This possibility was raised by studies showing that injection of neuraminidase-treated autologous IgG prompts the appearance of anti-IgG reactivity as well as by the demonstration of plasma neuraminidase activity and increased free sialic acid levels in patients with APSGN. Another potential cause of anti-Ig reactivity is the binding of IgG to type II receptors in the streptococcal wall. Anti-IgG reactivity is a constant feature of injections of streptococci cultured in a medium with autologous serum.

Additional autoimmune phenomena have been found in APSGN patients. Anti-DNA and anti-Ciq antibodies along with anti-neutrophil cytoplasmic antibodies (ANCA) can sometimes be present. Interestingly, ANCA has been found in up to 70% of ASPGN patients with azotemia or crescentic glomerulonephritis [3]. An autoimmune hemolytic anemia secondary to anti-cardiolipin antibody has also been reported [20].

Genetic Aspects A genetic aspect to disease susceptibility has been suggested by a higher rate of disease in siblings than rates seen in the general population during epidemics [59]. APSGN has also been associated with HLA-DR4 and DR-1 [54] and genetic mutations in the complement factor H (CFH) gene have been suggested as a cause for inducing dense deposit disease after an episode of PSGN [51] as well as a chronic glomerulonephritis triggered initially by streptococcal infection [66].

Clinical Characteristics

Children from 4–14 years old are most frequently affected by APSGN, and it is infrequently seen below the age of 2 years or above the age of 20 years. Boys develop APSGN with twice the frequency of girls. APSGN usually follows a respiratory or skin streptococcal infection, though any site of infection can be a potential trigger. Anecdotally, greater latency between infection and nephritis is reported after skin infection (3–6 weeks) than respiratory infection (1–2 weeks).

During the latent period, asymptomatic children may have microscopic hematuria. The subsequent clinical course of ASPGN varies from a subclinical disease to an acute nephritic syndrome, with an accompanying nephrotic syndrome or a rapidly progressive crescentic glomerulonephritis also potentially present [54]. Although subclinical disease with hypocomplementemia and microhematuria is clinically asymptomatic, it can be accompanied by increased blood pressure. In epidemic conditions, it has been estimated that subclinical disease occurs 1.5 times more frequently than clinically apparent disease [2]. In non-epidemic situations, prospective studies of household members of index cases have shown that asymptomatic patients are up to five times more frequent than symptomatic patients [17, 59].

The most typical symptomatic clinical picture in APSGN is the acute nephritic syndrome (hematuria, edema, hypertension, and moderate proteinuria). Glomerular hematuria is an almost universal finding and gross hematuria is present in one-third of patients. On urinalysis red cell casts are present in association with dysmorphic red cells. Macroscopic hematuria usually disappears within days, but microscopic hematuria may persist for a year or more and can exacerbate during illness and more rarely after strenuous exercise.

Edema is seen in up to 90% of symptomatic children, with anasarca infrequent outside of younger children and ascites uncommon except with concomitant nephrotic syndrome. Hypertension is present in 60–80% of the children and is severe enough to require specific antihypertensive treatment in about half of the cases. Oliguria on presentation is reported in up to half of symptomatic children. Other nonspecific symptoms include general malaise, nausea, weakness, headache, and dull lumbar pain.

Edema and hypertension typically abate within the first few weeks. Persistent heavy proteinuria is a risk factor for progression to chronic kidney disease. Although alterations of GFR with azotemia occur in 25–30% of patients, the need for dialysis is infrequent. A rapidly progressive course occurs in less than 1% of the cases and when present is due to the development of crescentic glomerulonephritis.

Pathophysiology of the Acute Nephritic Syndrome The acute nephritic syndrome stems from the inflammatory reaction in the glomeruli. Although renal blood flow is usually normal, the filtration fraction can be depressed to below 1%. While the reduction in glomerular filtration has long been assigned a central role in any fluid retention with an acute glomerulonephritis, modulation of tubular sodium and water handling including changes in the expression of the epithelial sodium channels and increased intrarenal angiotensin activity has been described [26], [28], [60].

Sodium and water retention results in expansion of the extracellular volume, with ensuing edema and hypertension. Plasma levels of renin, angiotensin, aldosterone, and atrial natriuretic peptide reflect an expanded extracellular volume, with the extent of suppression of plasma renin activity and aldosterone and the stimulation of atrial natriuretic peptide correlated with the severity of edema. Renal prostaglandin and kallikrein activities are also suppressed [54].

As glomerular inflammation decreases, the factors promoting volume retention moderate. The subsequent development of spontaneous or induced diuresis results in reduction in edema and correction of high blood pressure.

Serological Findings With APSGN the most constant serological finding is a reduction in serum complement levels that occurs in more than 90% of cases. The complement system is usually activated via the alternative complement pathway with reduced C3 complement levels as well as C1 and C4 complement. The classical and the lectin pathways of complement activation may also be engaged, however, and there are no clinical characteristics associated to a specific complement pathway's activation. Complement levels return to normal usually within 1 to 3 months after the development of acute glomerulonephritis, reflecting the tendency for a rapid spontaneous improvement in disease activity.

IgG and IgM serum levels are elevated in 80–90% of patients with APSGN. An elevated anti-streptococcal antibody titer is the usual laboratory confirmation of a preceding streptococcal infection since positive cultures are obtained in only 20–25% of cases, except during epidemics. Anti-zSPEB/SPEB serum titers have been found to be the best markers of nephritogenic streptococcal infections in multicenter studies based in Latin America [44], but these tests are not widely available to most clinicians, and anti-streptolysin O and anti-DNAse B titers are most frequently assayed. Two-thirds of ASPGN patients in the first week of disease may also manifest cryoglobulins and elevated IgG-anti-IgG rheumatoid factor titers, though again these studies are less commonly assessed [54].

Pathology Renal biopsy is seldom done in patients with APSGN. Biopsy is indicated when specific features raise the possibility of a different diagnosis, for instance if the serum complement is normal at presentation or stays persistently depressed several months after diagnosis. In the setting of rapid progression of kidney failure and concern for crescentic glomerulonephritis, the diagnosis also needs biopsy confirmation.

Light Microscopic Findings In APSGN, light microscopy usually reveals a diffuse proliferative glomerulonephritis (GN). The glomeruli are often enlarged and show global endocapillary hypercellularity. Some cases will show focal endocapillary proliferation or just mesangial proliferation [37]. In the acute phase of the disease, the GN also tends to be exudative, with a

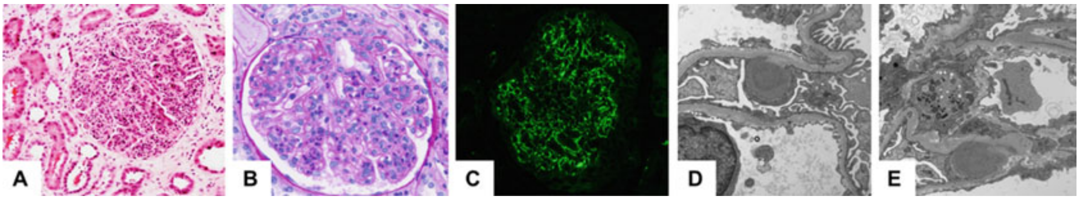

Fig. 1 **Pathology of Acute post-streptococcal glomerulonephritis**. (**a**). Acute post-streptococcal glomerulonephritis (APSGN) with severe proliferative and exudative GN. The glomerulus is enlarged and markedly hypercellular with a large number of neutrophils. Note the red blood cells in some tubular lumens. Hematoxylin and eosin stain, original magnification x200. (**b**). The glomerulus shows endocapillary hypercellularity with multiple neutrophils, although far fewer than the glomerulus in Fig. 1. Periodic acid-Schiff stain, original magnification x400. (**c**). Immunofluorescence staining for C3 in APSGN. There is granular staining in the glomerular capillary walls and mesangium, in a "starry-sky" pattern. Fluorescein isothiocyanate conjugated anti-human C3, original magnification x400. (**d**). Electron-microscopy in APSGN. Note the large, "hump"-like subepithelial deposit. The periphery of this deposit is slightly less electron-dense than its center, indicating very early resorption. The glomerular capillary with the subepithelial "hump" also contains a small subendothelial deposit. The glomerular basement membranes themselves are unremarkable and the podocyte foot processes are partially effaced. Uranyl acetate and lead citrate stain, original magnification x7500. (**e**). Electron microscopy in subacute post-streptococcal GN. A large, "hump"-like subepithelial deposit is present in a mesangial waist region; deposits are also present within the adjacent mesangial matrix. Uranyl acetate and lead citrate stain, original magnification x6000.

variable but oftentimes large number of neutrophils, as shown in Figs. 1a and 1b. After the first few weeks of disease, there is a progressive decline in cellularity, initially from the loss of the neutrophils, and a predominant mesangial proliferation persists.

In the acute phase of the disease, there is also often interstitial inflammation, which is typically comprised of a mixture of lymphocytes, monocytes, plasma cells, and neutrophils. Histologic evidence of acute tubular injury, characterized by the flattening of proximal tubular epithelium with loss of brush borders and nuclear enlargement, is also common. In adults, mild to moderate arteriosclerosis is generally seen, and in cases with underlying diabetic nephropathy, severe arteriosclerosis with arteriolar hyalinization and thickening can be found [37].

Immunofluorescence Microscopy Immunofluorescence findings in evolving APSGN have been elegantly defined [63]. In the first few weeks of the disease, fine granular deposits of C3 and usually IgG are found in the glomerular capillary walls as well as in mesangial areas, in what has been termed a "starry sky" pattern (Fig. 1c). Later in the disease, with resorption of many of the capillary wall deposits, there is a predominantly mesangial pattern of staining with a predominance of C3. Coarse to confluent granular staining along the glomerular capillary walls in what is termed the "garland" pattern can also be seen [63]. It is this pattern that best shows the individual subepithelial "humps" that are the characteristic ultrastructural feature of this disease.

In both acute and subacute post-streptococcal GN, deposits of C3 are invariably present, accompanied by IgG in most cases, and by IgM in approximately 50%, although the latter staining tends to be of low intensity [37, 61]. IgA staining is uncommon and of low intensity when present, although IgA is often the dominant immunoglobulin present in post-staphylococcal GN [22, 37, 36, 61]. C1q staining, typically of low intensity, can be seen in approximately one-third of cases [37]. Staining for light chains tends to follow the findings for IgG, with similar staining intensity for both kappa and lambda. If crescents are present, blotchy fibrinogen staining is typically noted at the periphery of the glomerular tuft and in a focal and segmental pattern.

Electron Microscopy The characteristic ultrastructural findings of APSGN are large subepithelial electron-dense deposits with a "hump-like" appearance, as shown in Fig. 1d. The number of these deposits varies considerably, and they can be segmentally limited or rather numerous, although not so common as to mimic a membranous nephropathy. The size of the subepithelial "humps" also varies considerably within any given glomerulus. In early postinfectious lesions, these deposits tend to be concentrated at or near the glomerular basement membrane reflection over mesangial areas, what is called the mesangial "waist" or "notch" (Fig. 1e) [23]. In resolving cases, the fraction of subepithelial deposits localized to mesangial waist areas increases further as more peripheral deposits are resorbed ([21]; [37]; [63]). Mesangial deposits are also abundant in the great majority of APSGN cases, as are subendothelial deposits though these tend to be smaller and more segmental in distribution ([37]; [61]). Extraglomerular deposits are not a feature of this disease.

Diagnosis

The typical clinical presentation is the sudden onset of dark urine with the development of swelling of the face and legs, oliguria, and hypertension. A history of a preceding upper respiratory or skin infection suggests a poststreptococcal etiology that may be confirmed by a positive streptococcal culture or rising anti-streptococcal antibody titers.

Positive streptococcal cultures are frequent in epidemic conditions but are found in fewer than 25% of sporadic cases. Throat swabs for streptococcal culture should be done in the setting of symptomatic pharyngitis, but are otherwise of limited utility. Evidence of a previous streptococcal infection usually depends on the demonstration of an elevated titer of serum antistreptococcal antibodies. Anti-streptolysin O and anti-DNAse B titers are the most frequently elevated in upper respiratory infections and impetigo, respectively. The streptozyme test which includes four antigens (DNAse B, Streptolysin O, hyaluronidase, and streptokinase) is reported to be positive in more than 80% of the cases.

Since a preceding infection may have been subclinical or so mild as to not be recalled, although ASPGN is the most common reason for a nephritic syndrome in a child, when there is not a compelling history of preceding illness, it is important as part of the initial diagnostic approach to assess if the acute nephritic syndrome is associated with a systemic condition with other manifestations or if it results from a primary renal disease. Determining complement levels allows there to be a relatively rapid determination if there is a hypocomplementemic GN, in which case conditions such as lupus nephritis, shunt nephritis, endocarditis, and the C3 glomerulopathies may also need to be considered in addition to APSGN. Normal complement levels and the nephritic syndrome can be found with IgA nephropathy and Henoch-Schonlein purpura, hemolytic uremic syndrome, vasculitis, and anti-glomerular basement membrane disease. In APSGN, serum complement levels return to normal quickly, often within a month and almost always within 3 months. Persistence of low complement levels should raise suspicion for a diagnosis different from APSGN and may prompt a kidney biopsy.

Treatment

Antibiotic Treatment If streptococcal infection is present at the time of diagnosis, it should be treated with penicillin or some other effective antibiotic. In epidemic situations and in high-risk settings, the administration of preventive antibiotic treatment to household members of index cases has been shown to decrease the number of subsequent APSGN cases [25].

Early administration of penicillin to infected children is reported to prevent or ameliorate the severity of acute glomerulonephritis, and at least one report suggests that APSGN patients who receive antibiotic treatment have a milder clinical course [75]. If active infection is not apparent at the time of diagnosis, antibiotic treatment is generally not provided to a child with APSGN. Since cross infection of household members and siblings of index cases can be very high, there does need to be attention to screening any symptomatic individual and treating with antibiotics if indicated [59]. If throat swab streptococcal culture is not readily available for symptomatic index cases or their symptomatic contacts, then empiric antibiotic provision is often provided.

Therapy with penicillin provided intramuscularly or orally is usually adequate treatment. Erythromycin is typically the treatment of choice in patients allergic to penicillin, although it should be noted that the existence of erythromycin-resistant strains may be increasing in many areas of the world, so a knowledge of local antibiotic-resistance patterns can help determine most effective empiric therapy ([12, 52, 53, 64].

Prophylactic antibiotic treatment is indicated in epidemic situations and may be considered in household members of actively infected index cases in non-epidemic conditions. Serologic evidence of recent streptococcal infection can be high in household members, and some reports have noted that in some situations up to one-third of household members may manifest some degree of nephritis [2, 54]. Especially in high risk communities, this strategy of treating household contacts may result in fewer cases of APSGN [25].

Treatment of the Acute Nephritic Syndrome Historically, children with APSGN often were made to restrict physical activity, including prolonged periods of bedrest. There is no evidence to support bed rest or activity restriction, especially if the blood pressure is not severely elevated and the child is feeling well.

To help prevent exacerbation of volume overload, sodium and fluid restriction are the most important initial steps for any child with acute nephritic syndrome. Loop diuretic provision can accelerate the resolution of edema and improve hypertension [50] whereas thiazide diuretics are generally far less effective. Oftentimes, diuretic therapy is needed for only a very short period of time.

In terms of blood pressure therapy, vasodilator therapy with calcium channel blockers or with hydralazine can be effective for significant blood pressure elevation. Aldosterone antagonists carry the risk of hyperkalemia, especially if there is any reduction in GFR. Similarly, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers also carry the risk of hyperkalemia or exacerbating any reduction in GFR.

On occasion, continuous infusion of a calcium channel blocker, nitroprusside, or labetalol may be needed to treat a hypertensive urgency or emergency. Posterior reversible leukoencephalopathy syndrome (PRES) has been described complicating APSGN and can present with mental disturbances, visual hallucinations, headache, and convulsions [1]. PRES requires head MRI examination to confirm its diagnosis and rule out other causes of encephalopathy.

Pulmonary edema may occasionally complicate the clinical course, and if unresponsive to diuretic therapy and other medical maneuvers may necessitate urgent dialysis for ultrafiltration. Dialysis may also be needed for those children with severe azotemia, hyperkalemia, or acidosis that does not respond to other medical therapy, as well as with children with significant loss of kidney function as occurs with a rapidly progressive glomerulonephritis (RPGN) as a consequence of APSGN.

With an RPGN picture and a kidney biopsy with crescentic change to a significant number of glomeruli, many clinicians will provide pulse dose methylprednisolone intravenously followed by some course of high dose oral steroids that are gradually tapered. No randomized trials exist to support this approach, and this management stems from historic general approach to RPGN prior to the ability to discern a specific diagnosis through biopsy or serology. There is also no evidence to support other immunomodulatory therapy either acutely or long-term in APSGN, especially if there is rapid improvement in kidney failure. Although an immune-mediated pneumonitis has been described in PSGN [71], this is thought to be exceedingly rare, and no other immunologic anomalies requiring immunosuppressive therapy have been described.

Prognosis

Most children recover quickly from APSGN, with significant initial medical morbidity or mortality usually a result of hyperkalemia or pulmonary edema early in the disease course. Long-term prognosis is also generally good, though as with any illness that predisposes to acute kidney injury, there is some risk of chronic kidney disease.

Initial studies in the first half of the twentieth century reported complete recovery in affected children, but the follow-up periods were short. Subsequent observations found that abnormal urinary findings could be seen during follow-up in anywhere from 3.5% to 60% ([6, 49]. A recent report found that 20% of patients followed for 10–20 years after APSGN manifested an abnormal urinalysis, though GFR decline occurred in fewer than 1% [57]. Another study of 110 Chinese children followed for 15–18 years after presentation reported non-nephrotic proteinuria in 7.2%, microhematuria in 5.4%, hypertension in 3%, and altered GFR in 0.9% [44]. Studies in Australian communities of indigenous people indicate that APSGN patients have an increased risk on follow-up for albuminuria (adjusted odds ratio 6.1, 95% CI 2.2–16.9) and hematuria (OR 3.7, CI 1.8–8.0) in comparison to controls who did not have APSGN [57].

The long-term kidney prognosis after APSGN may be significantly influenced by any other risk factors for chronic kidney disease that an affected child may hold. For instance, the superimposition of APSGN on conditions such as diabetes and metabolic syndrome have been postulated to play a role on the high incidence of end stage kidney disease in some communities of indigenous people in Northern Australia [70]. It has also been reported that persisting arterial stiffness, as determined by brachial–ankle pulse wave velocity, is more common in patients with who develop chronic kidney disease after APSGN [73].

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Infections That Affect the Kidney (Nonviral)

26

Arpana Iyengar and Vera Koch

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Abstract

The pathogenesis of infection-related renal complications in children is multifactorial and includes direct invasion of renal parenchyma, stimulation of an immune reaction, injury to the capillary endothelium, induction of a humoral response, or nephrotoxicity associated with the therapy of choice. This chapter presents non-streptococcal bacterial, fungal, and parasitic infections, some of which occur predominantly in tropical and subtropical regions, but which have been increasingly diagnosed in other regions of the globe on account of travel mobility.

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Keywords

Tropical · Bacterial · Parasitic · Fungal · Infections · Child

Introduction

In this chapter, we will limit the discussion to non-streptococcal bacterial infections that include staphylococcal infection associated glomerulonephritis (SAGN) and other infections such as endocarditis, shunt nephritis, and visceral abscesses. Viral infection-related kidney involvement, post-streptococcal glomerulonephritis, and central venous catheter-related infections are described elsewhere.

Bacterial Infections

Renal involvement secondary to bacterial infection is multifactorial in nature and includes direct invasion of renal parenchyma, stimulation of an immune reaction, injury to the capillary endothelium, or induction of a humoral response (Fig. 1). Monocyte activation is triggered by bacterial infections (e.g., post-infectious glomerulonephritis) that stimulate T-cell and B-cell responses, leading to immune complex-mediated glomerular injury. Inflammatory responses, secondary to infection can cause interstitial injury and acute tubular necrosis (e.g., leptospirosis). Mechanisms for renal ischemia and acute tubular necrosis in bacterial infections include hypovolemia, endothelial injury, disseminated intravascular coagulation, hemolysis, and complement activation [1].

Tropical Infections

Tropical infections are important contributors to the burden of acute kidney injury (AKI) mainly affecting the continents of South America and South Asia [2, 3]. Organ involvement including the kidney is underreported despite the risk of mortality and morbidity associated with these infections. There is a need to increase awareness of these infections and their sequelae as these regions could continue to face the challenges of climate change, water and sanitation issues, antimicrobial resistance, and delay in diagnosis or treatment, leading to a potential risk for chronic kidney disease (CKD) development.

Tropical bacterial and protozoal infections can manifest with urinary abnormalities and AKI. Leptospirosis, malaria, dengue illness, scrub typhus, and bacterial gastroenteritis are notable infections associated with renal involvement in the tropics. These infections commonly present as a febrile illness with a wide spectrum of other systematic features including jaundice, rash, splenomegaly, myalgia, respiratory symptoms, and thrombocytopenia [3]. The notable tropical bacterial infections, their renal involvement, and key renal pathological features are listed in Table 1 [1, 4–7, 12–14].

Seasonal changes can contribute to infections. A spectrum of bacterial infections causing AKI in adults during the monsoon season in India was noted to be acute gastroenteritis (23%), leptospirosis (13%), and typhoid fever (3.5%). In children, a study in South India revealed non-cholera diarrhea (7.8%), cholera (3%), typhoid fever (2.4%), scrub typhus (1.8%), tuberculosis (1.2%), and leptospirosis (0.6%) to cause AKI [8, 9].

Acute gastroenteritis was the most common diagnosis for AKI (44–60%) in hospitalized children from Argentina and Uganda [10]. However, the precise organism (bacterial/viral) causing diarrheal disease is commonly not evaluated in most developing countries. The reader is directed to the chapter on hemolytic uremic syndrome for details on bacterial diarrheal disease caused by *Escherichia coli*, Shigellosis, and *Campylobacter* species.

Tuberculosis (TB)

Latent Tuberculosis: Latent TB is a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens with no evidence of clinically active TB. Immunosuppression from any cause including prolonged steroid therapy, nephrotic syndrome, or chronic kidney disease is a potential risk factor for reactivation. The World Health Organization (WHO) recommends three

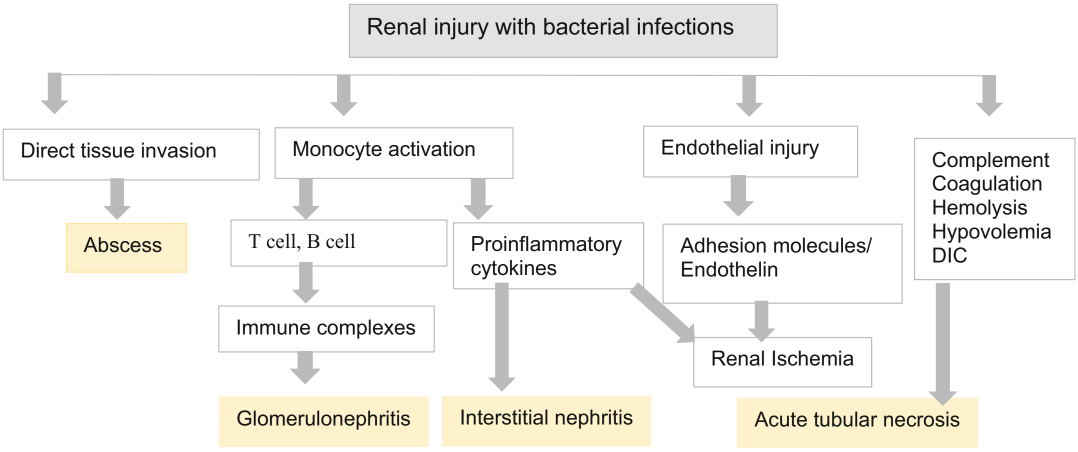
Renal injury with bacterial infections
Direct tissue invasion
Abscess
Monocyte activation
T cell, B cell
Immune complexes
Glomerulonephritis
Proinflammatory cytokines
Interstitial nephritis
Endothelial injury
Adhesion molecules/ Endothelin
Renal Ischemia
Complement
Coagulation
Hemolysis
Hypovolemia
DIC
Acute tubular necrosis

Fig. 1 Mechanisms of renal injury due to bacterial infections. (Adapted from reference [1])

Table 1 Profile of renal involvement and renal pathology findings in common bacterial tropical infections. (Adapted from reference [1])

Infection	Abnormal urine sediment	Proteinuria	AKI	CKD	Predominant renal pathological findings
Tropical bacterial infections					
Leptospirosis	++++	++++	+++	+	ATN, AIN
Tuberculosis	++	++	–	+	AIN
Scrub typhus	+	++	+	–	ATN, AIN, MPGN
Salmonellosis	+++	++++	+	–	AIN, MPGN, nephritis, and pyelonephritis
Diphtheria [4]	+	+	+	–	AIN, ATN
Cholera [5]	–	–	+	–	ATN
Tetanus [6]	+	++++	+++	–	ATN
Leprosy [7]	++	+++	+	+	ATN,MPGN,MN, amyloidosis
Syphilis [8–11]	+++		+		MN, MPGN, RPGN
Legionellosis			++		Rhabdomyolysis, AIN, ATN, proliferative glomerulonephritis

ATN: acute tubular necrosis; AIN: acute interstitial nephritis; MPGN: membranoproliferative glomerulonephritis; MN: membranous nephropathy; RPGN: rapidly progressive glomerulonephritis; +: less common; ++++: very common

tests to detect latent TB: the tuberculin skin test/ Mantoux test and two Interferon-Gamma release assays – QuantiFERON-TB Gold In-Tube and T-SPOT TB. Positive tests cannot differentiate latent TB from active TB, so an evaluation to exclude active TB is warranted prior to considering treatment. Options for treating latent TB are monotherapy with Isoniazid for 6 months in countries with high and low TB incidence or alternatively, rifampicin plus Isoniazid daily for 3 months for children and adolescents aged <15 years in countries with a high TB incidence [15].

Renal and Urinary TB: Amongst those affected with genitourinary TB, children form a very small proportion (<5%) and manifest only after the first decade of life. Genitourinary TB is classified based on the extent of anatomical involvement and spans through nondestructive forms of parenchymal TB, TB papillitis, cavernous renal TB, and urinary tract TB [16]. Reactivation of *Mycobacterium tuberculosis* primary infection, especially in an immunocompromised patient, leads to a tubercle or granuloma formation that progresses to caseating necrosis and dissemination of bacilli into the collecting system and urinary tract. Unlike other bacterial urinary tract infections, tuberculous infection of the urinary tract takes a descending route. The latent period for reactivation following a primary infection ranges from 9 to 33 years.

The most common site is unilateral kidney followed by ureter and bladder involvement [17]. Renal involvement can be in the form of localized disease or secondary to miliary TB with tubercles in the renal cortex. Granuloma formation can begin in the cortex, adjacent to glomeruli or the peritubular capillaries but are less distinct in patients with associated HIV infection or immunosuppressed patients. The renal medulla is the preferred site for colonization of *M. tuberculosis* bacteria and during reactivation can lead to granuloma formation, caseation necrosis, papillitis, and papillary necrosis. Granulomatous chronic interstitial nephritis that is composed of epithelioid macrophages, lymphocytes, and Langhans giant cells (Fig. 2) can destroy the medullary compartment. The kidney parenchyma becomes replaced by caseous necrosis leading to a "putty kidney" [18]. Rarely, renal TB can present as crescentic glomerulonephritis, membranous nephropathy, or associated with amyloidosis and chronic kidney disease [19, 20].

Lower urinary tract symptoms, constitutional symptoms, gross hematuria, recurrent urinary infections, and sterile pyuria are common manifestations of urinary TB [21]. Ureteric strictures, bladder fibrosis, and renal calcification are known consequences of tuberculosis [22, 23]. Chronic fibrosis of the bladder wall and vesicoureteral junction can lead to the classical *"thimble bladder"* appearance and *"golf hole" ureter,* respectively (Fig. 2b, c).

Diagnosis: Isolation of *M. tuberculosis* in urine samples or histopathologic evidence of TB in any relevant tissue specimen confirms the diagnosis. Radio imaging plays a critical role in the diagnosis of urinary tuberculosis. Acid-fast bacilli smear microscopy of urine has high specificity but low sensitivity [24]. Culture techniques with Mycobacteria Growth Indicator Tube (MGIT) yields high specificity with acceptable sensitivity but is time consuming. The Xpert MTB/RIF is a cartridge-based nucleic acid amplification test (NAAT) for rapid diagnosis of urine *M. tuberculosis* DNA and antibiotic sensitivity. Another rapid diagnostic test is the lateral flow assay that detects mycobacterial cell wall glycolipid lipoarabinomannan (LAM) [25, 26].

Management: The principles of management include antimycobacterial cure and preservation

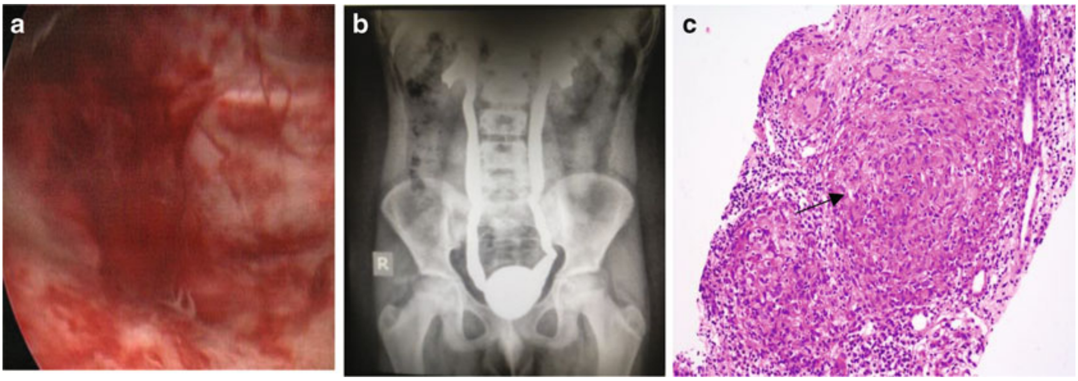

Fig. 2 (**a**) Cystitis cystica changes of the bladder wall with multiple areas of diffuse mucosal bleeding in an adolescent boy diagnosed with urinary tuberculosis. (**b**) The MCU reveals a small capacity bladder with patulous ureteric orifices (Thimble bladder) and bilateral vesicoureteral reflux. (**c**) Light microscopy of the kidney specimen showing granulomatous inflammation(black arrow) composed of epithelioid macrophages, lymphocytes, and langhans giant cells replacing a large portion of the tubulointerstitial compartment. A glomerulus in the lower right corner is unremarkable. H&E X200. (Courtesy of Dr. Anila Abraham, Renopath, Centre for renal and urological pathology Pvt. Ltd. Chennai, India)

of tissue or organ function. Anti-tuberculous treatment for newly diagnosed genitourinary TB is for 6 months duration consisting of an *intensive phase* (2 months) with Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol followed by *continuous phase* (4 months) with Isoniazid and Rifampicin. In those previously treated for tuberculosis, the regimen is for 8 months with Isoniazid, Rifampicin, Pyrazinamide, Ethambutol, and Streptomycin (2 months) followed by Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol (1 month) and Isoniazid, Rifampicin, and Ethambutol (5 months) [27, 28]. Guidelines on management of multidrug resistant TB, an emerging challenge, has been made available by the WHO [29].

A recent review on urogenital TB describes the approach to medical and surgical management [30]. Surgical therapies for genitourinary TB are indicated in complicated disease. Nephrectomy is indicated for nonfunctioning kidney with or without calcification and in the presence of extensive disease involving the whole kidney, in those with hypertension with ureteropelvic junction (UPJ) obstruction and pyonephrosis. Some of the reconstructive surgeries that are undertaken for complicated urinary TB are ureteroneocystostomy, stricture excision, augmentation cystoplasty, and urinary conduit diversion.

Leptospirosis

Leptospirosis is a zoonotic, waterborne disease caused by *Leptospira interrogans*, an obligate aerobic spirochete. It is a re-emerging disease, endemic to tropical and subtropical regions but unfortunately underreported and underrecognized [31]. Primary reservoirs are rodents or rats. The kidney is commonly affected in humans with leptospiral infection. Spirochetes colonize the proximal convoluted tubule and are excreted in the urine of the rat [32]. In humans, spirochetes adhere to tubular epithelium, invade the renal interstitium, and get excreted in the tubular lumen over a span of 1–2 weeks.

Risk factors for leptospirosis are related to occupational exposure, namely, agriculturists, animal caretakers, fisherman, sewage workers, mining workers, and sugarcane workers. A contaminated environment has been identified as a risk factor in urban slum populations [33, 34]. Leptospirosis can manifest as an early anicteric phase (3–7 days) with fever, headache, myalgia, nausea, vomiting, malaise, and conjunctival redness. This is followed by a temporary recovery phase that leads to the second phase (icteric phase) characterized by multiorgan involvement. Weil's disease is a severe manifestation of leptospiral infection that manifests with hepatic failure, pulmonary hemorrhage, and acute kidney injury [35].

During infection, the activated innate immune system generates an inflammatory response. The microbial Pathogen-Associated Molecular Patterns (PAMPs) get recognized by the Pattern Recognition Receptors (PRRs) expressed at the surface of Toll-like receptors (TLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). This triggers release of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα). An uncontrolled cytokine production could result in a cytokine storm that leads to sepsis-like syndrome with multiorgan failure. The outer membrane proteins (OMP) of *Leptospira* contain antigenic components causing tubular dysfunction and inflammation. The OMP LipL32 binds to TLR2 in proximal tubule cells activating the nuclear factor and production of iNOS and TNF-α in the proximal tubule [36–38].

The spectrum of renal involvement in leptospirosis ranges from asymptomatic urinary abnormalities of mild proteinuria and microscopic hematuria to AKI, hemolytic uremic syndrome-like disease, and CKD of unknown origin. AKI is a common complication of leptospirosis in both the icteric and anicteric phases of the disease. The prevalence of acute kidney injury is reported to be 10–60% in studies from Brazil and South Asia [39]. In hospitalized patients, leptospirosis is responsible for AKI in 0.6% of children and 45.8% of adults [40, 41]. Leptospirosis can coexist with other infections, such as scrub typhus [42], Dengue infection [43], and Hanta virus [44], that can complicate the clinical presentation and outcome.

Renal involvement in leptospirosis has been reviewed comprehensively [39, 45, 46] and acute tubulointerstitial nephritis is the most common underlying pathological lesion. The primary site of injury is the proximal convoluted tubule leading to a decrease in sodium/hydrogen exchanger isoform 3 (NH3), aquaporin 1 and 2, and Na^+ K^+ ATPase [47]. Tubulointerstitial damage is also a consequence of hemodynamic alterations, hypotension, hyperbilirubinemia, and rhabdomyolysis [32]. Glomerular lesions are rare and could manifest with mild proteinuria secondary to foot process fusion and focal swelling of the basement membrane [48]. *Leptospira* associated AKI manifests as a non-oliguric AKI accompanied by hypokalemia; tubular proteinuria; and sodium, magnesium, and phosphate wasting. However, oliguric AKI has also been reported in children [49]. Hyperkalemia may be seen with severe AKI or rhabdomyolysis and is a predictor of poor outcome [32]. AKI in the majority resolves by the second week of disease and mortality is lower in children compared to adults [50].

Diagnosis

The urinary sediment usually contains red blood cells (RBC), white blood cells, bile casts, and granular casts. Dark field microscopy of urine shows spirochetes between the first and fourth week of illness. Leptospiral organisms are fragile, which makes isolation from the blood, cerebrospinal fluid, or urine challenging. Microagglutination tests are highly sensitive and specific to detect *Leptospira*-specific antibodies. A fourfold rise in bacterial cell titer in samples collected 2 weeks apart confirms infection. In the first week of illness, rapid antibody tests using an enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin M (IgM) to leptospiral antigens are useful. The real-time quantitative Polymerase Chain Reaction (PCR) assay is a sensitive method for detection of leptospiral DNA. Several molecular methods for serotyping of leptospires are available [32, 51]. Recent reviews propose newer biomarkers for AKI in various tropical infections, including malaria, leishmaniasis, and schistosomiasis. Biomarkers for leptospiral AKI are NGAL, Syndecan-1, and ICAM-1 [52, 53].

Renal biopsy (Fig. 3) findings involve a spectrum of acute interstitial nephritis, acute tubular necrosis with bile or red blood cell casts, mesangioproliferative glomerulonephritis or focal necrotizing glomerulonephritis, and, rarely, vasculitis with focal hemorrhage [45, 54].

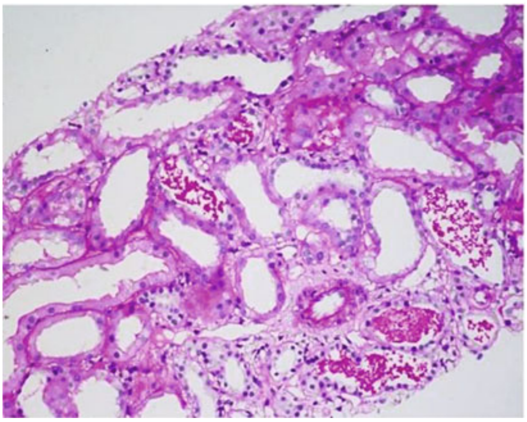

Fig. 3 Acute tubular injury due to leptospirosis. Periodic acid–Schiff (PAS), ×200. Tubular epithelial cells are attenuated and the lumen is expanded. Cell debris is seen in some of the tubules. Immunostaining for myoglobin was negative. There is interstitial edema. The glomeruli in this biopsy specimen appeared to be morphologically normal. Immunofluorescence staining for immunoglobulin (Ig)G, IgM, IgA, complement component (C) 3 (C3), C1q and kappa and lambda light chains were negative. (Images are courtesy of Dr. A Abraham, Renopath, Center for Renal and Urological Pathology Pvt., Chennai, India)

Treatment

Severe leptospirosis is treated with intravenous penicillin, ceftriaxone, or cefotaxime for 7 days. Oral antibiotics, such as doxycycline, amoxicillin, ampicillin, erythromycin, or azythromycin, are indicated in less severe disease [55]. Supportive therapy is critical in the form of fluid resuscitation, maintenance of acid–base and electrolyte balance, and respiratory support. Doxycycline in high risk individuals has been found to be effective in preventing leptospiral infection [56]. Dialysis is indicated in about 60% of adults with severe leptospiral AKI. Reduction in mortality has been observed with early daily dialysis compared to late and alternate day dialysis [45, 57]. Though hemodialysis and hemofiltration are associated with lower mortality and shorter time of recovery in adults, there is no consensus on the best modality of dialysis in children. Various modalities of dialysis, such as peritoneal dialysis, hemodialysis, and hemofiltration, have been used [45, 58]. Plasmapheresis has been found to be effective in severe forms of leptospiral AKI [59].

Outcomes: The WHO reports pediatric mortality rates due to leptospirosis of 0.22–0.32 per million age-related population, with AKI being an important predictor of poor outcome [60]. Risk factors for death include AKI, oliguria, pulmonary involvement, leukocytosis, thrombocytopenia, and electrocardiographic abnormalities [50, 61]. Leptospiral infection could lead to chronic tubulointerstitial nephritis and cause progression of kidney diseases insidiously. It has been proposed to be a risk factor for CKD of unknown origin [62, 63].

Rickettsia

Rickettsial infections are a group of zoonoses causing febrile illness in the tropics and transmitted by ticks, fleas, or mites. Fever, rash or eschar, conjunctival congestion, myalgia, hepatosplenomegaly, and systemic involvement are common manifestations [64]. Rickettsial diseases have a wide distribution and vary in severity, from self-limited infections to fulminant and often fatal illnesses. Scrub typhus, Rocky mountain spotted fever, and Q fever are important infections that involve the kidney.

The spectrum of renal pathology with rickettsial infections includes acute interstitial nephritis, acute tubular necrosis, vasculitis, rhabdomyolysis, direct invasion of tubules, and, rarely, acute glomerulonephritis [65, 66]. Scrub typhus is caused by the bacterium *Orientia tsutsugamushi* (*Rickettsia tsutsugamushi*) endemic to South Asia, and is transmitted to humans by infected Leptotrombidium mite larva. In adults, the prevalence of associated urinary abnormalities is high with 20–60% presenting with AKI, and oliguria, thrombocytopenia, and rhabdomyolysis predict AKI [67, 68]. In children, the burden of AKI secondary to scrub typhus ranges from 3% to 16%, including those from intensive care units [40, 69]. The mechanisms of AKI are attributed to direct infiltration, associated glucose-6-phosphate dehydrogenase deficiency, rhabdomyolysis, and renal ischemia leading on to ATN, interstitial nephritis (Fig. 4), and mild mesangial glomerulonephritis. Early treatment with doxycycline has been effective in clinical and renal recovery [66, 70, 71].

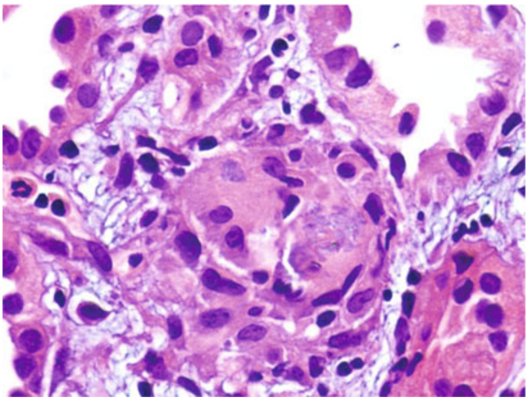

Fig. 4 Acute tubulointerstitial nephritis secondary to scrub typhus. Light microscopy shows acute tubular injury with crystal formation and edematous interstitial space in the vicinity with occasional inflammatory cells (HE stain, 40x). (Courtesy of Dr. M Vankalakunti, Pathology Lab, Manipal Hospitals, Bangalore, India)

Renal involvement in Q fever caused by *Coxiella burnetii* includes asymptomatic hematuria, proteinuria, acute tubulointerstitial nephritis, or immune-mediated glomerulonephritis [72, 73]. Nearly one-third of children with Rocky Mountain spotted fever caused by *Rickettsia rickettsii* are affected with AKI causing a higher risk of mortality [74–76]. While Weil Felix test has poor sensitivity as a diagnostic test, PCR assays are sensitive for diagnosis of rickettsial infections. Doxycycline is the drug of choice and chloramphenicol and macrolide antibiotics are alternative options. Supportive management is the mainstay of management of AKI [64].

Infection-Related Glomerulonephritis

Infection-related glomerulonephritis (IRGN) is a term used to describe glomerulonephritis that is associated with ongoing infection (syn-infectious) or that which occurs with a latent period following an infection (post-infectious GN) [77].

In the past decade, there have been several good reviews on IRGN, post-infectious glomerulonephritis (PIGN), and *Staphylococcus*-

associated GN [78–81]. In this regard, the differences in the clinical presentation of IRGN between adults and children have also been highlighted [82]. Moreover, the understanding of the various mechanisms related to infections triggering glomerulonephritis has considerably improved. The proposed key mechanisms described are based on the theory that there is a hereditable genetic risk factor (first hit) that predisposes individuals to mount an altered autoimmune response. This is followed by exposure to infectious agents (second hit) that could get modified by epigenetic factors leading to activation of the innate immune system alongside the antigen-specific adaptive immune response. This in turn generates antigen-specific T cells and B cells that mediate glomerular injury through the inflammatory effector cells or complement system. Similarly, in staphylococcal associated GN, nephritogenic antigens like the super antigens of *Staphylococcus* could trigger immune complex entrapment within the glomeruli or immune complexes formation in situ against planted glomerular antigens. Additionally, the alternate/lecithin pathway and activated plasmin and complement pathways act in concert to cause tissue injury [82, 83].

Non-streptococcal bacteria-associated glomerulonephritis mainly includes staphylococcal and other bacteria-related (intravascular and extravascular) glomerulonephritis (Fig. 5). *Staphylococcus*-infection-associated glomerulonephritis (SAGN) is an entity that has emerged as one of the important causes for glomerulonephritis mainly in adults. While this specific entity is uncommon in children, staphylococcal endocarditis and shunt infection-related GN are not uncommon. From a broader perspective, it becomes important to differentiate the different types of IRGN. Key differences in the clinical features, investigations, pathology, and outcomes between PSGN and SAGN are listed in the Table 2. It also is important to differentiate IgA dominant IRGN from primary IgAN. The points in favor of the former are presence of hypocomplementemia, renal dysfunction, endocapillary proliferative GN, IgA deposits in the capillary walls, and subepithelial humps(Fig. 6).

A culture proven staphylococcal infection (Methicillin-resistant *Staphylococcus aureus* and Methicillin sensitive *S. aureus* and coagulase-negative *S. epidermidis* are the common organisms) is the definitive criteria for diagnosis. Supportive care along with antibiotic therapy forms the main stay of treatment for SAGN. Immunosuppression should be avoided as it does not have a clear benefit in these patients, and renal sequelae post recovery from infection is variable.

Glomerulonephritis Associated with Bacterial Infections: Endocarditis/Shunt Nephritis

Focal bacterial infections can involve the kidney. There has been an evolution over time in the renal involvement of systemic infections, with a change in the spectrum from streptococcal to a broader group of bacteria including staphylococcus. Besides respiratory and gastrointestinal infections, the two specific entities of focal bacterial

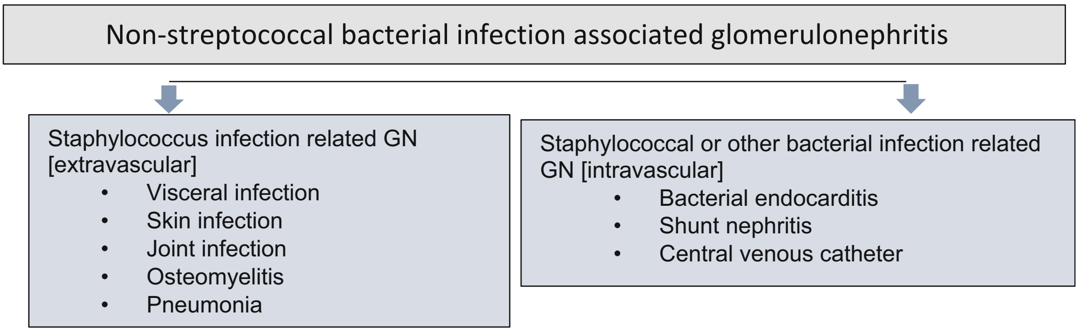
Non-streptococcal bacterial infection associated glomerulonephritis
Staphylococcus infection related GN [extravascular]
• Visceral infection
• Skin infection
• Joint infection
• Osteomyelitis
• Pneumonia
Staphylococcal or other bacterial infection related GN [intravascular]
• Bacterial endocarditis
• Shunt nephritis
• Central venous catheter

Fig. 5 The clinical entities associated with non-streptococcal glomerulonephritis. (Adapted from Refs. [81, 84])

Table 2 Differences between PSGN and SAGN. (Adapted from reference [77])

Parameters	PSGN	SAGN
Age	Mainly children	Mainly adults, occasionally children
Infection source	Pharyngitis, pyoderma, otitis media, tonsillitis, infected scabies	Endocarditis, skin abscess, leg ulcers, osteomyelitis, septic arthritis, pneumonia
Infection free latent period	Present	Not present
Clinical features	Hematuria, hypertension, oliguria, mild proteinuria, occasionally renal dysfunction	AKI, microscopic hematuria, nephrotic range proteinuria, occasionally leukocytoclastic vasculitis rash
Laboratory features	Low C3, normal C4	Low C3 in 30–50%, normal C4
Renal histopathology	LM: Diffuse exudative proliferative GN	LM: Mesangial hypercellularity, endocapillary proliferation in some, segmental necrotizing lesions common, acute tubular necrosis frequent
	IF:C3 with or without IgG along capillary loops and mesangium EM: Predominantly subepithelial deposits	IF: IgA dominant or codominant with C3 or exclusive C3, pauci-immune in a few. EM: Predominantly mesangial deposits
Outcomes	Usually complete recovery	Unpredictable

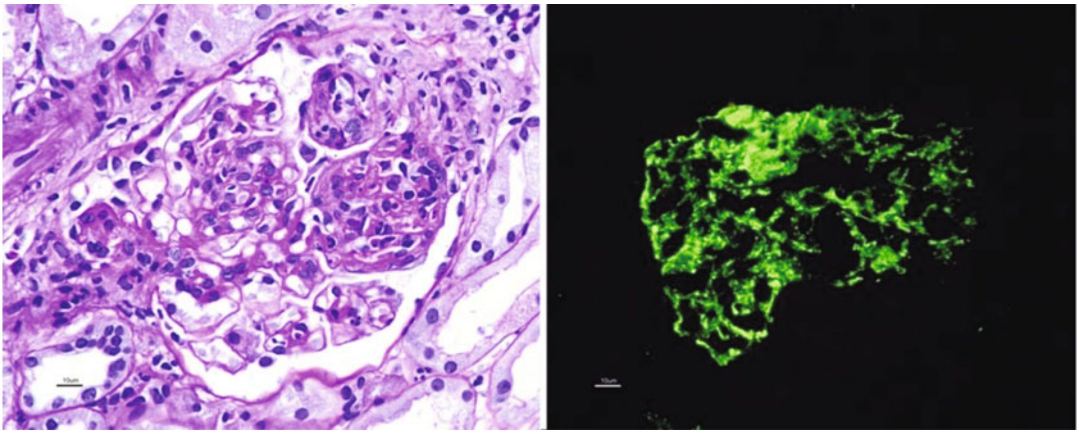

Fig. 6 Light microscopy shows global endocapillary proliferation with presence of neutrophils in the tufts. Cellular crescent is noted partially (PAS stain, 40x). Immunofluorescence shows coarse granular deposits in the mesangial region and capillary walls with IgA (3+). (Courtesy: Dr. M Vankalakunti, Nephopathologist, Manipal Hospitals, Bangalore, India)

infections causing renal injury are endocarditis and shunt nephritis.

Bacterial Endocarditis

The reported occurrence of renal lesions in patients with bacterial endocarditis is variable. Renal manifestations in patients who underwent renal biopsies ranged from 18% to 25% [85, 86]. Common bacteria causing endocarditis are *Staphylococcus*, *Streptococcus*, and *Bartonella henselae*, occasionally rickettsial infection, while culture negative endocarditis is not uncommon. Children at risk for endocarditis are those with valvular heart disease and congenital heart disease and rarely adults with vascular access-related bacteremia [87, 88].

Clearly there has been a shift in the paradigm of the pathogenesis behind renal involvement in bacterial endocarditis over time. Historical reports revealed microemboli from infected vegetations to cause renal lesions but ongoing evidence proposed immunologic mechanisms to be the pathogenic factor. However, there still prevails uncertainty in the evidence that supported immune complex mechanisms. The alternative mechanisms proposed are direct injury of endothelial cells by bacteria, trigger of antibodies against self-protein like proteinase 3 causing ANCA antibody-associated autoimmune renal injury, and abnormalities in complement regulatory proteins [89, 90].

Clinical presentation: In a cohort of 49 patients with endocarditis, including two children, the most common clinical syndromes of renal involvement were acute renal failure, acute nephritic syndrome, rapidly progressive glomerulonephritis, and nephrotic syndrome. However, severe AKI and rapidly progressing glomerulonephritis associated with bacterial endocarditis are rare entities in children [88, 91, 92]. Hematuria is seen in the majority with reduction in C3 levels seen in over 50% of patients. In a minority, low C4 and ANCA serology is positive. In recent years there has been appreciable number of case reports describing subacute bacterial endocarditis (*Streptococcus*/*Enterococcus*/*Bartonella*) associated with ANCA-PR3 positivity and pauci-immune GN [93–95].

The underlying histopathological lesions range from a spectrum of renal infarction due to septic emboli, acute interstitial nephritis, and cortical necrosis in the early reports to crescentic glomerulonephritis, diffuse proliferative glomerulonephritis, mesangial hypercellularity, and acute tubular injury with no micro-abscess or cortical necrosis among recent reports [86, 88]. C3 deposits in IF predominate followed by IgM and fewer IgG and IgA have been observed. Rarely, pauci-immune staining and full house under IF have been identified. Immunoglobulin and C3 deposits were associated with *Bartonella* and *Coxiella* infection-related endocarditis and there were no specific differences in the pathology features of streptococcal and staphylococcal endocarditis. Electron microscopy typically reveals electron dense deposits in the mesangial area and subendothelial space.

Treatment and outcomes: Treatment primarily consists on appropriate antibiotics and immunosuppression in cases of severe renal injury. Methylprednisolone, cyclophosphamide, and plasmapheresis have been used in various case reports in adult and children. In a predominant adult series, 21% deaths were observed. In those who survived, 32% had complete renal recovery and 10% went on to renal failure. Case reports of children also reveal use of aggressive immunosuppression in selected cases of severe renal dysfunction alongside antibiotics with variable outcomes of recovery or persistent renal sequelae [88, 89, 91, 92].

Shunt Nephritis

Shunt nephritis is an uncommon immune complex-mediated nephritic syndrome with or without renal dysfunction that is associated with chronically infected cerebrospinal fluid shunts. The common shunts undertaken are ventriculoatrial, ventriculoperitoneal, ventriculo-jugular, and, recently, endoscopic third ventriculostomy that have been reported across a wide range of patients from infants to the elderly. Though culture negative shunt infections are common, predominant bacteria reported to cause infections are coagulase negative *Staphylococcus* and *Propionibacterium acnes* [96, 97].

The time to develop nephritis following shunt infection is widely variable ranging from weeks to years. Pathogenesis involves formation of immune complexes triggered by the bacterial antigen that get deposited in the glomeruli and activate the complement system. Clinically patients present with fever, hematuria, hypertension, proteinuria, hepatosplenomegaly, skin rash, arthralgia, and anemia. Further, hypocomplementemia, elevated mixed cryoglobulins, and, rarely, positive ANCA have been reported [96, 98]. Histopathology reveals mesangial hypercellularity and features of membranoproliferative glomerulonephritis with mesangial deposits of IgM and C3 under IF and subendothelial deposits under EM. The treatment involves antibiotics therapy with or without shunt removal surgery. Renal recovery is associated with early and prompt removal of shunt or replacing a ventriculo-atrial shunt with a ventriculoperitoneal shunt. In the recent years there have been case reports mainly of adults highlighting the importance of early diagnosis of shunt nephritis and possible renal sequelae [97, 99, 100]. In a pooled review on the outcomes of shunt nephritis amongst 76 patients (43 children), 54% had complete renal recovery, 18% had persistent urinary abnormalities, 19% progression to renal failure, and deaths occurred in 9% [101].

Parasitic Infections

Malaria

Epidemiology

Malaria transmission occurs predominantly through the bites of female *Anopheles* mosquitoes, 30 among more than 400 different species of *Anopheles* mosquito; are the most important malaria vectors. The vector species bite between dusk and dawn. The intensity of transmission is dependent on characteristics of the parasite, the vector, the human host, and the environment. *Anopheles* mosquitoes lay their eggs in water, which hatch into larvae. Adult female mosquitoes look for a blood meal to nurture their eggs. Each species of *Anopheles* mosquito has its preferred aquatic habitat; the rainy season in tropical countries favors the formation of small water puddles, which facilitate mosquito proliferation (https://www.who.int/news-room/fact-sheets/detail/malaria, accessed on 7/20/20 and 1/2/2022).

Places where the mosquito lifespan is longer and human bites are preferred over other animals are ideal for transmission of the disease, as exemplified by Africa, responsible for approximately 90% of the world's malaria cases on account of the long lifespan and strong human-biting habit of its vector species. Climatic conditions such as rainfall patterns, temperature, and humidity also affect transmission, which may be seasonal, with the peak during and just after the rainy season. Malaria epidemics can occur secondary to climate change in areas where people have little or no immunity to malaria or when people with low immunity move into high transmission areas of malaria, to find work, or as refugees (https://www.who.int/news-room/fact-sheets/detail/malaria, accessed on 7/20/20 and 1/2/2022).

The development of human immunity to malaria depends on the length and intensity of exposure. In high transmission areas, years of exposure may lead to partial immunity in adults. Although partial immunity does not provide complete protection, it reduces the risk that malaria infection will cause severe disease. Children who are exposed to the disease very early in life and individuals of all age groups of areas with less transmission and low immunity do not benefit from partial immunity and are at risk of severe disease (https://www.who.int/news-room/fact-sheets/detail/malaria, accessed on 7/20/20 and 1/2/2022).

Human disease may be caused by five species of *Plasmodium* parasites: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae, Plasmodium ovale*, and *Plasmodium knowlesi* [102]. *Plasmodium falciparum* is the most prevalent malaria parasite in the WHO African Region, accounting for 99.7% of estimated malaria cases in 2018, 50% of the cases in the WHO South-East Asia Region,71% cases in the WHO Eastern Mediterranean Region, and 65% cases in the WHO Western Pacific Region. Globally, 53% of the *P. vivax* burden is in the WHO South-East Asia Region, with the majority being in India (47%). *P. vivax is responsible for 75%* of the malaria cases in the WHO Region of the Americas (https://www.who.int/news-room/feature-stories/detail/world-malaria-report-2019, access on July 20, 2020 and 1/2/2022).

The life cycle of the five *Plasmodium* species is similar, except from the dormant stages of *P. ovale* and *P. vivax* [103]. After inoculation by an infectious *Anopheles* mosquito, sporozoites invade hepatocytes. After a period of 1–2 weeks of asexual replication, initially in the liver, thousands of merozoites are released into the blood per infected hepatocyte, Merozoites invade erythrocytes, where they repeat the process of asexual reproduction, the erythrocytes undergo rupture, and the newly formed merozoites continue the cycle of invasion and replication. The repeated cycles of asexual replication within erythrocytes are associated with the clinical symptoms of malaria. Some of the parasites in the blood develop into male and female gametocytes whose ingestion by a female *Anopheles* mosquito is a key step for malaria transmission.

Between 2010 and 2018 the incidence rate of malaria declined globally from 71 to 57 cases per 1000 population at risk. In 2018, most malaria cases and 94% of all malaria deaths occurred in the WHO African region. In 2018, children under 5 years of age proved to be the most vulnerable group and accounted for 67% (272,000) of all malaria deaths worldwide (https://www.who.int/news-room/feature-stories/detail/world-malaria-report-2019, access on July 20, 2020 and 1/2/2022).

Clinical Features of Malaria

Clinical disease can be classified as *uncomplicated* or *severe malaria*.

Uncomplicated Malaria

Uncomplicated malaria may be caused by any of the human infecting *Plasmodium* species. It usually presents as a nonspecific febrile illness with symptoms and/or signs of malaria and a positive parasitological test, in the absence of evidence of end organ damage. Fever, an almost universal sign of the disease, is often higher than 40 °C and associated with rigors, chills, and intense perspiration as it resolves.

Other common nonspecific initial symptoms include malaise, fatigue, headache, cough, abdominal pain, anorexia, nausea, vomiting, diarrhea, myalgia, and back pain, and their recognition as symptoms of malaria is dependent on a high index of suspicion, which is fundamental in the care of any possibly exposed individual. Hemolysis and splenic enlargement may lead to mild jaundice. Laboratory abnormalities may include mild anemia, thrombocytopenia, mild coagulopathy, elevated blood urea nitrogen, and a small creatinine elevation, not compatible with acute kidney injury criteria [103]. The current prevalence of sickle hemoglobin trait, in many tropical populations, may reflect selection for the sickle cell trait [HbAS] through a survival advantage against death from falciparum malaria. Although HbAS seems to have no effect on the prevalence of symptomless parasitemia, it may be associated with 50% protection against mild clinical malaria, 75% protection against admission to the hospital for malaria, and almost 90% protection against severe or complicated malaria. The effect of HbAS on episodes of clinical malaria was mirrored in its effect on parasite densities during such episodes [104].

Severe malaria is generally caused by adherence to capillary beds of multiple organs of erythrocytes infected by *P. falciparum*. In high-transmission areas, young children and visitors of any age from nonendemic areas present with the higher risk of developing severe falciparum malaria. A list of clinical and laboratory characteristics of severe malaria is shown in Table 3. The diagnosis of severe malaria is defined by the presence of one or more of the listed clinical and laboratory aspects, which may occur alone or in combination, and a positive malaria diagnostic test in the absence of an optional cause.

Severe malaria may present as one or more of the following syndromes [105].

1. Cerebral malaria: develops because of parasite adherence to cerebral vasculature, may present as altered mental status, coma, seizures, and evidence of increased intracranial pressure
2. Blackwater fever: an association of acute renal failure and hemoglobinuria secondary to intravascular hemolysis
3. Severe anemia: associated with high parasite burden leading to intense hemolysis of infected red blood cells and defined as hemoglobin less than 7 g/dL
4. Acute respiratory distress syndrome
5. Hyperparasitemia: defined as presence of malaria parasite infection of greater than 5% of circulating erythrocytes
6. Metabolic complications: defined by findings such as severe metabolic acidosis and life-threatening hypoglycemia

Renal Involvement in Malaria

Severe malaria can cause disease in glomeruli, tubules, and in the kidney interstitium [106].

Malarial AKI

Acute kidney injury (AKI) is a relatively frequent complication of severe malaria and may be associated with the infection by *P. falciparum, P. vivax,*

Table 3 Clinical and laboratory manifestations of severe malaria (http://www.who.int/malaria/publications/atoz/9789241549127/en)

Clinical features of severe malaria
Impaired consciousness (including unarousable coma)
Prostration, i.e., generalized weakness so that the patient is unable to sit, stand, or walk without assistance
Multiple convulsions: more than two episodes within 24 h
Deep breathing and respiratory distress (acidotic breathing)
Acute pulmonary edema and acute respiratory distress syndrome
Circulatory collapse or shock; systolic blood pressure <80 mm Hg in adults and <50 mm Hg in children
Acute kidney injury
Clinical jaundice plus evidence of other vital organ dysfunction; and
Abnormal bleeding
Laboratory and other findings
Hypoglycemia (<2.2 mmol/l or <40 mg/dl)
Metabolic acidosis (plasma bicarbonate <15 mmol/l)
Severe normocytic anemia (hemoglobin <5 g/dl, packed cell volume <15% in children; <20% in adults)
Hemoglobinuria;
Hyperlactatemia (lactate >5 mmol/l); renal impairment (serum creatinine >265 μmol/l)
Pulmonary edema (radiological).: Radiologically confirmed or oxygen saturation less than 92% on room air with a respiratory rate greater than 30/min, often with chest indrawing and crepitations on auscultation
Hyperparasitemia: *P. falciparum* or *P. knowlesi* parasitemia greater than 10% (CDC uses >5% as a cutoff)

P. malariae, and *P. ovale*. Its incidence in the pediatric population varies depending on the choice of AKI definition and the characteristics of the studied cohort [106]. Adult literature and pediatric studies describe the association of malarial AKI with increased mortality. The pathogenesis of malarial AKI is incompletely understood; the three currently accepted hypotheses favor its association with hemodynamic, immune-mediated glomerular injury, and metabolic alterations [107].

The hemodynamic hypothesis states that malarial AKI is primarily caused by hemodynamic changes secondary to adherence from parasitized erythrocytes to adjacent healthy erythrocytes, platelets, and the capillary endothelium (cytoadherence) with the formation of intravascular clusters and rosettes. At the kidney level, the infected erythrocytes may disrupt the deep vascular beds microcirculation, leading to anemia, thrombocytopenia, and impaired blood flow to the kidneys promoting tissue hypoxia. Hypovolemia secondary to insensible fluid losses caused by fever, tachypnea, low oral intake, or vomiting may add to the hemodynamic compromise resulting in acute tubular necrosis, the most frequent cause of malarial AKI [107].

Cortical necrosis, also described in malaria, characterizes a more severe kidney injury generally associated with future development of renal failure. Its predisposing factors include hypovolemia, vasoconstriction, hemolysis/hemoglobinuria, erythrocyte parasitemia, immune complexes deposition in glomeruli, microcirculation dysfunction due to cytoadherence of infected erythrocytes, and, more rarely, rhabdomyolysis [106].

Hemoglobinuria may cause direct renal tubular injury. “Blackwater fever” is a clinical syndrome that associates hemolysis-related hemoglobinuria to AKI. Erythrocyte destruction may be related to malarial infection or to antimalarial drug toxicity, especially in patients with G6PD deficiency. Rarely, myoglobinuria from associated rhabdomyolysis may affect tubular function [108]. Thrombotic microangiopathy (TMA) has been described in association with malarial AKI. It may be related to microvascular disruption by cytoadherence or it may be secondary to antimalarial drug toxicity, especially after exposure to mefloquine and quinine agents [2].

Immune dysregulation is the second proposed mechanism for malarial AKI. Peripheral blood mononuclear cells would be activated by surface antigens on host erythrocytes and parasites' cells, leading to the release of proinflammatory cytokines such as TNF-α and interleukins 1, 6, 8, and γ. Immunofluorescence might show mesangial and capillary wall deposition of immune complexes of malarial antigen, IgG, IgM, and C3. Autoantibodies such as anticardiolipin and antiphospholipid antibodies have been associated with malarial immune-mediated vascular pathology [107].

The third proposed mechanism of malarial AKI is metabolic disruption. Parasitic infection would affect the erythrocyte cell membrane and interfere with the erythrocyte magnesium-activated ATPase. Therefore, the intracellular sodium content would be reduced creating an increased calcium influx into the cell, leading to an increased red cell deformability, shortened lifespan, and an increased tendency to peripheral pooling and organ sequestration. Other potential metabolic causes of AKI in malarial infection must include renal damage from oxidative stress caused by cell-free hemoglobin, from significant hemolysis of both healthy and infected erythrocytes. Cell-free hemoglobin is a mediator of erythrocyte membrane phospholipid peroxidation leading to changes in the red cell deformability and promoting production of prostaglandin isomers (F2-IsoP and IsoFs) which act as potent renal vasoconstrictors reducing renal blood flow and renal function [107].

Another contributing factor for kidney disease in malaria may be liver dysfunction. The main described scenarios are cast nephropathy secondary to hyperbilirubinemia and hepatorenal syndrome [106, 107]. Malaria, especially in patients with repeated episodes of infection, may lead to chronic kidney disease [106].

Clinical Features of Malarial AKI

Non-oliguric AKI may develop in up to 80% of patients affected by malaria usually 3–7 days after the onset of fever. It usually improves in 17 ± 6 days. Oliguric cases may persist for weeks [2]. Lactic acidosis secondary to tissue hypoxia, hyponatremia, and hyperkalemia are rather frequent electrolyte abnormalities observed in these patients. Hyponatremia develops before the onset of oliguria due to the initial internal dilution and sodium wasting. Hyperkalemia is secondary to hemolysis, acidosis, and, rarely, rhabdomyolysis. Patients with malarial AKI may develop proteinuria due to glomerulonephritis [2, 106, 108].

Treatment of Malarial AKI

As with AKI treatment in non-malarial infection, supportive care is the basis for treatment of malarial AKI. Data from the *Fluid Expansion as Supportive Therapy (FEAST)* trial of fluid resuscitation in children with severe infection demonstrated an increase in 48 h mortality in patients who were given fluid boluses in comparison with those who were not. A subgroup analysis of children with and without malaria showed that fluid boluses were of no benefit to either group [109]. The study concluded that, unless the patient presents with significant hypotension, fluid boluses should be avoided in the treatment of malarial AKI. Continuous IV fluids may be used with careful patient monitoring for signs of fluid overload [107].

In patients with moderate-to-severe malaria, randomized control trials have demonstrated a reduction in the development of AKI with IV prostacyclin and with acetaminophen [108, 110].

The persistence of hemolytic anemia and thrombocytopenia post adequate malaria treatment demands a high index of suspicion for TMA, especially if medications such as quinine were used for treatment [2, 111].

An increased expression of KIM-1 and matrix metalloproteinase-3 (MMP-3) in tissues from autopsied patients with malaria-associated AKI has been confirmed. Chitinase-3-like 1 (CHI3L1) was associated to AKI in children with severe malaria, with evidence to its association with mortality. Initial data show that approximately 31% of patients with malaria-associated AKI had normal levels of creatinine at presentation, illustrating the importance of new AKI biomarkers [112].

Current evidence suggests that coma, evidence of metabolic alterations such as acidosis and hypoglycemia, and renal failure are the most consistent and important predictors of death in pediatric *P. falciparum* malaria [113].

Malarial Glomerular Disease

Malarial glomerulopathy occurs rather infrequently in association with *P. malariae* and *P. falciparum*. Malarial nephritides are currently understood because of endothelial damage and immune-complex deposition. The disruption of the microvasculature homeostasis by intravascular hemolysis, coagulation, and local release of inflammatory mediators may lead to glomerular endothelium activation and damage. The presence of glomerular deposits of immunoglobulins and of circulating immune complexes points to the probable involvement of humoral immunity in the development in malarial nephritis. Hyperimmunoglobulinemia and autoantibodies are frequent findings in patients with malaria. As the presence of immune complexes containing malarial antigens is demonstrated in a fraction of the glomerular immune deposits, polyclonal B-cell activation is suggested. A generalized T-cell immunosuppression secondary to the production of immunosuppressive cytokines by macrophages and/or T cells is also observed and could be important in the development of polyclonal B-cell activation [114].

Renal involvement in the course of *P. malariae* infection (quartan malaria) presents generally with nephrotic syndrome. The most frequently observed type of glomerular lesion is a membranoproliferative type of glomerulonephritis with sparse proliferation of endothelial and mesangial cells. Granular deposits of gM, IgG, and C3 are observed in mesangial and subendothelial areas by immunofluorescence. Findings of focal and segmental glomerulosclerosis and global glomerulosclerosis may be present. Chronic glomerular disease associated with *P. malariae* is usually irreversible with treatment of the infection, suggesting a role for genetic and environmental factors [114].

Diagnosis and Treatment of Malaria

The WHO recommends prompt malaria diagnosis either by microscopy or malaria rapid diagnostic test (RDT) in all patients with suspected malaria, before administration of treatment (https://www.who.int/malaria/publications/atoz/9789241549127/en, last access on 10/24/2020/).

Given potentially severe complications of malarial infection, current treatment efforts focus on prevention. There is currently no commercially available malaria vaccine. The complexity of the malaria parasite makes development of a malaria vaccine an exceedingly difficult task. Recent progress has been made with the completion of a Phase 3 trial of the RTS, S/AS01 candidate vaccine and review by the European Medicines Agency and WHO. Over 20 other vaccine constructs are currently being evaluated in clinical trials or are in advanced preclinical development (https://www.who.int/immunization/research/development/malaria/en/, accessed on 7/22/20 and 1/2/2022).

Filaria

Epidemiology

Lymphatic filariasis is a mosquito-borne neglected tropical disease that can be caused by infection from three species of nematode parasites: *Wuchereria bancrofti*, responsible for 90% of the cases, *Brugia malayi*, and, more rarely, by *Brugia timori*. It is transmitted by five genera of mosquitoes (*Culex*, *Anopheles*, *Aedes*, *Mansonia*, and *Ochlerotatus*). Adult worms dwell in the lymphatic vessels and disrupt the normal function of the lymphatic system. The worms can live for approximately 6–8 years and, during their lifetime, produce millions of microfilariae or immature larvae that circulate in the blood.

Mosquitoes are infected with microfilariae by ingesting blood when ingesting a blood meal from an infected host. Microfilariae develop into infective larvae within the mosquito. Mature parasite larvae enter the body after being deposited on the skin during the infected mosquitos' bite. The larvae migrate to the lymphatic vessels where they develop into adult worms, continuing the transmission cycle.

In 2000, the WHO launched the *Global Program to Eliminate Lymphatic Filariasis* (GPELF). WHO's strategy has focused on stopping the spread of infection through annual large-scale provision of the recommended basic package of care and vector control to all eligible people in affected areas (https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis, last access on 10/24/2020).

Clinical Presentation and Genitourinary Involvement in Filariasis

Lymphatic filariasis infection may be asymptomatic, or present as acute or chronic conditions. The majority of infections are asymptomatic, affected individuals contribute to transmission of the parasite, without external signs of disease. When lymphatic filariasis develops into chronic conditions, it presents as lymphedema or elephantiasis of limbs and hydrocele. Breasts and genital organs are frequently involved. Chyluria and tropical pulmonary eosinophilia are less common clinical conditions. Secondary bacterial skin infections, or less frequently the death of adult worms, often complicate chronic lymphedema or elephantiasis, leading to the development of acute episodes of skin, lymph nodes, and lymphatic vessel inflammation. Hydrocele is the most common chronic clinical abnormality in men with bancroftian filariasis. It results from the accumulation of clear straw-colored fluid in the tunica vaginalis surrounding the testicles. Genital manifestations such as edema of the labia majora are uncommon in females (https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis).

Chyluria, the presence of chyle in the urine, can be classified as either parasitic or nonparasitic. The primary cause of the parasitic form is filariasis. It is caused by rupture of dilated abdominal lymphatics into the urinary excretory system. It is generally episodic; each episode may last for days or weeks. Chyle or blood clots may cause urinary retention [115, 116]. Chyluria may be asymptomatic or present in association with dysuria, hematuria, anemia, hypoproteinemia, weight loss, malnutrition, and cachexia. Chyluric hypolipidemia may develop due to loss of lipids such as triglycerides, phospholipids, and cholesterol and deficiency of fat-soluble vitamins may occur. The differential diagnosis of chyluria includes cavitating renal tuberculosis, nephrotic syndrome, and retroperitoneal malignancies. Unlike patients with nephrotic syndrome, urinary sediment does not contain lipid-laden oval fat bodies or fatty casts but may present with large numbers of lymphocytes, consistent with the leakage of lymphatic fluid into the urine [115, 116].

Renal disease is rarely observed in patients without microfilaremia. Kidney disease can be diagnosed predominantly in areas with poor vector control and inadequate health-care facilities. Glomerular involvement shows a variety of lesions by light microscopy, including diffuse, membranoproliferative, minimal-change, chronic sclerosing glomerulonephritis, and the collapsing variant of focal segmental glomerulonephritis. Microfilariae may be found in the arterioles, glomerular and peritubular capillary lumina, tubules, and interstitium. Immune deposits containing worm antigens and structural components can be shown by immunofluorescence and electron microscopy. Proteinuria and/or hematuria has been detected in over 50% of cases with lymphatic filariasis; 25% showed glomerular proteinuria [117]. Hematuria may be caused by mechanical damage to glomeruli by circulating microfilariae or a manifestation of immune-mediated glomerulonephritis [118].

An increase or development of new onset proteinuria and worsening of kidney function can be observed following initiation of diethylcarbamazepine or ivermectin probably due to an exacerbation of the immune process secondary to antigen release into circulation after death of the parasite. The response to treatment is inconsistent in those with nephrotic syndrome; kidney function decline may continue, despite clearance of microfilariae. Therapeutic apheresis has been utilized to reduce the microfilarial load before starting diethylcarbamazepine to prevent antigen release [117].

Diagnosis

Circulating microfilariae can be detected by examining thick smears (20–60 μl) of fingerprick blood. Blood must be collected at a specific time depending on the periodicity of the microfilariae. Rapid blood antigenic and antibody diagnostic tests and microfilariae DNA detection in human blood and in mosquitoes using Polymerase Chain Reaction (PCR) have been recommended for mapping, monitoring, and transmission assessment surveys (https://www.cdc.gov/

parasites/lymphaticfilariasis/health_professionals/dxtx.html, last access 1/2/2022).

Treatment

Treatment of lymphatic filariasis is currently implemented using single-dose combination drug regimens, which generally include diethylcarbamazine (DEC). A single dose of albendazole (400 mg) with either DEC (6 mg/kg) or ivermectin (200 μg/kg) reduces microfilaria counts to very low levels for at least 24 months, in the absence of reinfection. Albendazole with DEC is believed to have better macrofilaricidal activity than albendazole with ivermectin. However, since DEC should not be used in patients with onchocerciasis, the albendazole/ivermectin regimen is preferred for treatment of lymphatic filariasis in areas that are co-endemic for onchocerciasis. Mass drug administration programs usually provide these medicines annually [115].

Onchocerca volvulus and *Loa loa*

Glomerular disease has been reported in association with two other filarial infections: *Loa loa* and *Onchocerca volvulus*

Onchocerciasis, also known as river blindness, is found in sub-Saharan Africa and sometimes in Central and South America. It is caused by a roundworm, *Onchocerca volvulus*. The main vectors for human onchocerciasis are the *Simulium* black flies. Skin involvement is common and presents as intense pruritus and inflammation resulting in papules, plaques, hyperpigmentation, and widespread lichenified onchodermatitis. Ocular involvement is also common and may result in blindness. A higher incidence of proteinuria and nephrotic syndrome, in association with *Onchocerca volvulus*, is observed in hyperendemic regions in Africa. Renal biopsy may show minimal change, mesangioproliferative, or chronic sclerosing glomerulonephritis. Onchocercal antigens as well as IgM, IgG, and C3 can be shown on immunofluorescence. Treatment with diethylcarbamazine may be effective in early glomerular lesions but usually fails to treat renal lesions in patients with nephrotic syndrome [119].

Loa loa is known as the "African eye-worm" as adult worms occasionally move across the eyes of infected individuals. Humans are infected by a vector of the genus *Chrysops*, mainly *C. silacea* and *C. dimidiate*. Loiasis only occurs in Africa, where transmission is confined to rainforest and swamp forest areas of Central and Western Africa, from western Uganda to eastern Benin. The pathogenesis of *L. loa* infection is poorly understood. Loiasis may be asymptomatic, infected individuals frequently present with hypereosinophilia and high antibody titers to filarial antigens. Angioedema and pruritus are the most common clinical manifestations. Adult worms may migrate across the eye under the bulbar conjunctiva or under the skin in otherwise asymptomatic individuals. Diethylcarbamazine is the drug of choice for loiasis when microfilaria counts are low. Ivermectin and albendazole are other therapeutic options. Several cases of membranous glomerulopathy and a single case of focal and segmental glomerulosclerosis have been reported in patients with loiasis [114].

Schistosomiasis

Epidemiology

Schistosomiasis is an acute and chronic parasitic disease caused by trematode worms of the genus *Schistosoma*. Agricultural and fishing populations, without access to safe drinking water and adequate sanitation, are particularly predisposed to this disease. At least 90% of those requiring treatment for schistosomiasis live in Africa (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, last access on 10/24/2020 and 1/2/2022).

There are two major forms of schistosomiasis – intestinal and urogenital. Intestinal schistosomiasis may be caused by *Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi, Schistosoma guineensis*, and *Schistosoma intercalatum*, while urogenital schistosomiasis is caused by *Schistosoma haematobium* (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, last access on 10/24/2020 and 1/2/2022).

Transmission occurs when people infected by schistosomiasis contaminate freshwater sources with excreta (feces or urine) containing parasite eggs. The eggs hatch in water and contaminate an intermediary host, the freshwater snail. The snail subsequently releases larval forms of the parasite, which penetrate the human skin during contact with infested water, initiating the infection process. In the body, the larvae develop into adult schistosomes. Adult worms dwell in the blood vessels where the females release eggs. Eggs may be eliminated in the feces or urine continuing the parasite's life cycle or become trapped in body tissues, causing immune reactions and progressive damage to organs. Schistosomiasis transmission has been reported from 78 countries, 52 endemic countries with moderate-to-high transmission require preventive treatment, which is known to reduce and prevent morbidity, and should be repeated over several years. Schistosomiasis is also a travel-related disease affecting visitors of endemic locations who come in contact with infested waters (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, last access on 10/24/2020 and 1/2/2022).

Clinical Features

Exposition to infested waters may lead to a pruritic rash due to cercarial dermatitis.

Acute schistosomiasis, also known as Katayama fever, usually presents 2–8 weeks after contact with infested water. Common clinical presentation includes fever, lethargy, malaise, and myalgia. Less common symptoms include cough, headache, anorexia, urticarial or papular rash, right upper quadrant pain, bloody diarrhea as well as focal neurological signs [120].

Symptomatic chronic schistosomiasis may present months to years after primary exposure, not necessarily with a clear previous history of acute schistosomiasis. Disease onset is generally insidious and reflects the level and primary location of the infection, the immune response to the eggs and the extent of systemic involvement. *S. mansoni, S. mekongi, S. intercalatum*, and *S. japonicum* cause intestinal tract and liver disease. *S. haematobium* rarely causes intestinal or liver disease but characteristically causes urinary tract disease or genital involvement. In men, schistosomiasis can cause involvement of the epididymis, spermatic cord, testicles, and prostate [121].

Morbidity during human schistosomiasis results from chronic immune stimulation by soluble egg antigens. The chronic forms of disease are characterized by granulomatous inflammation and elevated levels of pro-inflammatory cytokines. Eosinophilia is frequently observed. Natural or post-treatment worm death may slowly induce protective resistance to reinfection, through release of immunogens, which may effectively react with antigens expressed by incoming schistosomula or lead to a decrease in worm fecundity [122].

Pediatric Schistosomiasis

Exposure to infection occurs gradually, there is a progressive increase in infection intensity and prevalence as children grow up so that practically all children living in high transmission areas will have had contact with schistosome cercariae by age one. This reality calls for the need to include pediatric schistosomiasis in national control programs for adequate planning of therapeutic options and other operational strategies implementation. In a recent study in Zimbabwean with preschool-aged children an increase in anti-parasite IgM and IgE titers was shown 6 weeks after treatment with praziquantel and was associated with resistance to reinfection, demonstrating that development of naturally acquired immunity may be accelerated by treatment [123].

Renal Involvement

The prevalence of renal involvement in schistosomiasis varies according to the study population. The incidence of all forms of glomerular involvement is estimated in 5–6% and increases to 15% in the hepatosplenic form. Glomerulonephritis is described in 10–12% in autopsy studies. Proteinuria is reported in 20% of patients with *S. mansoni* infection [124].

Schistosomal glomerulopathy is more frequently diagnosed in young male patients with hepatosplenomegaly, although it has been described in the hepato-intestinal form. It can be initially asymptomatic or open only with hypocomplementemia. Presentation with nephritic syndrome and non-nephrotic proteinuria with or without microscopic hematuria is frequent. Renal insufficiency develops rarely. Subclinical glomerulonephritis may be present in the chronic forms of schistosomiasis, but the true incidence of this complication is not known [124].

In *S. mansoni*-infected patients, circulating antigens of the adult parasite and its eggs can be found in different fluids and tissues. *Schistosoma* antigen deposits can be detected in the glomeruli of patients with focal and segmental glomerulosclerosis and mesangial proliferative glomerulonephritis. There is a strong correlation between the presence of circulating antigens and glomerular disease development [124]. A classification for schistosomal glomerulopathy has been proposed in five categories, based on histopathological findings: mesangial proliferative glomerulonephritis, membranoproliferative glomerulonephritis, focal and segmental glomerulosclerosis, exudative glomerulonephritis, and amyloidosis [125]. Patients with the mesangial proliferative form may be asymptomatic, present with minimal urinary changes, such as mild proteinuria and hematuria, or develop nephritic syndrome or renal insufficiency. Membranoproliferative glomerulonephritis is associated with hypocomplementemia, with low levels of CH50, C3, and C4, suggesting activation of the classical complement pathway. An obligatory differential diagnosis in this condition is hepatitis virus B/C-associated Membranoproliferative glomerulonephritis. Focal and segmental glomerulosclerosis (FSGS), with the same histopathological findings as described in idiopathic FSGS, is the third more common glomerulopathy in schistosomiasis. Schistosomal FSGS may originate as primary FSGS, as observed in HIV, or as secondary FSGS, due to focal scaring caused by mesangioproliferative glomerulonephritis. Membranous disease, minimal change disease, amyloidosis, and IgA nephropathy have been less frequently observed in schistosomiasis, and their pathophysiologic mechanisms are still poorly understood [124].

Coinfection with *Salmonella* may lead to new-onset or worsening of nephrotic syndrome. Treatment of coexistent *Salmonella* infection has a favorable influence on the course of the glomerular disease with improvement in serum complement levels, creatinine clearance, and proteinuria following anti-*Salmonella* therapy alone or in combination with anti-schistosomal treatment. The prognosis of schistosomal glomerulopathy varies according to the histological form of the disease: minimal change, mesangioproliferative, focal proliferative, diffuse proliferative, and endocapillary forms present relatively good prognosis, provided sustained eradication of *Schistosoma* and *Salmonella* infection can be achieved, while focal segmental glomerulosclerosis or amyloidosis usually progress to renal failure despite treatment [117].

In patients with *S. haematobium* infection, hematuria and dysuria generally occur 3 to 4 months after primary infection and may be due to inflammation and ulceration in the bladder mucosa. In endemic areas, many children present microscopic hematuria, which can later become macroscopic. Polyps, hypertrophic nodules, and eggs deposition can be observed through cystoscopy. Granulomas, fibrosis, and calcifications in the vesicle wall may lead to vesicoureteral reflux and obstructive uropathy, bladder cancer and, less frequently, renal failure [124]. Irreversibility of schistosomal renal lesions may result from delayed diagnosis. Specific antiparasitic treatment and prompt initiation in the initial phases of the disease may alter the renal disease development or progression. Patients with proliferative forms do not respond to antiparasitic treatment or to immunosuppression.

In systematic review of community-based studies describing the prevalence of proteinuria, hematuria, abnormal imaging, or kidney dysfunction among children in sub-Saharan Africa, the findings of intensity of kidney damage and frequency of abnormal imaging varied widely as shown by the reported prevalence of proteinuria 32.5% (2.2–56.0%); hematuria 31.1% (0.6–67.0%); hydronephrosis 11.3% (0.0–38.0%); hydroureter 7.5% (0.0–26.4%); and major kidney abnormalities 0.1% (0.0–0.8%). Serum creatinine was reported in 4 out of 32 evaluated studies with insufficient detail to identify the prevalence of renal dysfunction. Most of the studies were performed in *Schistosoma haematobium* endemic areas. A lower prevalence of kidney disease was observed in the few studies from non-endemic areas. The study concludes that published data on pediatric kidney disease in sub-Saharan Africa is highly variable and dependent on *S. haematobium* prevalence [126].

Diagnosis

Schistosomiasis is diagnosed by detection of parasite eggs in stool or urine specimens. Antibodies and/or antigens detected in blood or urine samples are also indications of infection.

The eggs of intestinal schistosomiasis can be detected in fecal specimens by the Kato-Katz technique, which uses methylene blue-stained cellophane soaked in glycerin or glass slides. In *S. mansoni* transmission areas, the CCA (Circulating Cathodic Antigen) test can also be used. Urogenital schistosomiasis can be diagnosed using nylon, paper, or polycarbonate filters as the standard diagnostic technique. Children with *S. haematobium* frequently have microscopic hematuria detectable by chemical reagent strips (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, last access on 10/24/2020 and 1/2/2022).

For people living in non-endemic or low-transmission areas, serological and immunological tests may be useful in confirming exposure to infection and the need for thorough examination, treatment, and follow-up (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, last access on 10/24/2020 and 1/2/2022).

Prevention and Treatment

The control of schistosomiasis is based on large-scale treatment of at-risk population groups, which includes access to safe water, improved sanitation, hygiene education, and snail control (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, last access on 10/24/2020 and 1/2/2022).

The WHO strategy for schistosomiasis control focuses on reducing disease through the large-scale periodic, targeted treatment with praziquantel (preventive chemotherapy) of affected populations. The treatment-targeted groups are school-aged children and adults at risk such as fishermen, farmers, irrigation workers, and women whose domestic tasks bring them in contact with infested water in endemic areas. In school-age children, the frequency of treatment is determined by the prevalence of infection. Treatment of preschool aged children, although also recommended, cannot be included in current large-scale treatment programs for lack of suitable formulation of praziquantel. In high-transmission areas, treatment may have to be repeated every year for several years. The impact of control interventions should be determined by close monitoring. In a few countries, where there is low transmission, the focus should be on the interruption of the transmission of the disease (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, last access on 10/24/2020 and 1/2/2022).

Leishmaniasis

Epidemiology

Leishmaniasis is caused by a protozoa parasite from more than 20 *Leishmania* species. *Leishmania* parasites are transmitted through the bites of infected female sandflies. Over 90 sandfly species are known to transmit *Leishmania* parasites. The characteristics of the parasite and sandfly species, the local ecology of the transmission sites, current and past exposure of the human population to the parasite, and human behavior determine the epidemiology of leishmaniasis, Approximately 70 animal species, including humans, are natural reservoir hosts of *Leishmania* parasites. Some of the poorest people on earth are affected by the disease, which is associated with malnutrition and its immunological consequences, poor housing, and lack of financial resources. Leishmaniasis is linked to environmental changes such as deforestation, building of dams, irrigation schemes, and urbanization. Only a small fraction of those infected by *Leishmania* parasites will develop the disease, therefore, the term leishmaniasis describes the condition of becoming sick due to a *Leishmania* infection, not to being infected with the parasite.

There are three main forms of the disease (https://www.who.int/news-room/fact-sheets/detail/leishmaniasis, access on 10/24/20 and 1/2/2022).

Visceral leishmaniasis (VL), also known as kala-azar, is fatal if left untreated, clinical presentation is characterized by irregular episodes of fever, weight loss, hepatosplenomegaly, pancitopenia, and polyclonal hypergammaglobulinemia. It remains one of the top parasitic diseases with outbreak and mortality potential. In 2018, more than 95% of new cases reported to WHO occurred in 10 countries: Brazil, China, Ethiopia, India, Iraq, Kenya, Nepal, Somalia, South Sudan, and Sudan. Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis. It causes skin lesions, mainly ulcers, on exposed parts of the body, leaving lifelong scars and serious disability or stigma. In 2018, over 85% of new cases occurred in 10 countries: Afghanistan, Algeria, Bolivia, Brazil, Colombia, Iran (Islamic Republic of), Iraq, Pakistan, the Syrian Arab Republic, and Tunisia. It is estimated that between 600,000 to 1 million new cases occur worldwide annually. Mucocutaneous leishmaniasis leads to partial or complete destruction of mucous membranes of the nose, mouth, and throat. Over 90% of mucocutaneous leishmaniasis cases occur in Bolivia, Brazil, Ethiopia, and Peru.

Kidney Involvement

A recent study described 73 patients admitted with cutaneous leishmaniasis, 17 of which (23.2%) were diagnosed with acute kidney injury. The risk factors for AKI in this case series were advanced age, longer delay between symptom onset and hospital admission, and longer hospital stay. Complete renal function recovery was observed in 11 cases (64.7%) at the time of hospital discharge. Observed urinary abnormalities included proteinuria (4.1%), hematuria (4.1%), and leukocyturia (5.4%). Hypokalemia was found in 12.3% of cases. Other case series have shown urinary concentration deficit in 77% of cases and urinary acidification deficit in 40.5% patients; increased microalbuminuria was observed in 35% of patients with ACL before specific treatment, and in only 8% of patients after treatment [127].

Glomerular lesions are only observed with visceral leishmaniasis caused by *Leishmania donovani*; Cutaneous or mucocutaneous leishmaniasis are not associated with glomerular involvement. Kidney involvement in visceral leishmaniasis is usually mild and transitory; it may present as glomerular or tubular manifestations. The glomerulopathy is mainly attributed to immunocomplex deposition leading to mesangial cell proliferation, hematuria, and proteinuria. The glomerular histology may show a variety of patterns ranging from purely mesangioproliferative to membranoproliferative lesions, sometimes associated with focal and segmental collapse of capillary loops. Tubulointerstitial damage is generally present and consists of tubular degeneration and inflammatory infiltration. Amyloidosis may be a complication of this condition. Immunofluorescence may show IgG, IgM, IgA, and C3 in the mesangium and capillary loops. Electron microscopy may show irregular thickening of the glomerular basement membrane with subendothelial and subepithelial dense deposits. The association of hyperimmunoglobulinemia with high IgG levels, circulating immune complexes, and high titers of rheumatoid factor and cryoglobulin, with the presence of glomerular immunoglobulin deposition suggests the involvement of polyclonal B-cell activation and “classical” B-cell activation in the pathogenesis of leishmanial nephritis.

Tubular dysfunction associated with incomplete distal tubular acidosis, impaired renal concentration, and high urinary magnesium excretion has also been described in visceral leishmaniosis. Clinical manifestations may present as urine concentration and acidification defects, microalbuminuria, proteinuria, microscopic hematuria and leukocyturia, acute interstitial nephritis, nephritic and nephrotic syndrome, and acute renal injury. Possible causes for AKI in visceral leishmaniasis patients include drug toxicity, associated infections, hemodynamic abnormalities, and immune complex deposits. Hypokalemia, leukopenia, chills, and amphotericin B use have been described as risk factors for AKI in adults. In children, secondary infections have been considered a risk factor for AKI [127]. Other less frequent disorders have been described in visceral leishmaniasis, such as primary adrenal insufficiency and the syndrome of inappropriate ADH secretion. Electrolyte disturbances are common and include hyponatremia (94.6%), hypokalemia

(26%), hypochloremia (27.2%), hypocalcemia (32%), and hypomagnesemia (41.8%). Increased urinary excretion fraction of sodium, potassium, magnesium, chloride, calcium, inorganic phosphate, and uric acid has been frequently observed. The cure of the leishmanial infection, after specific treatment, favors a regression of the above described glomerular and tubular lesions [114, 127].

Children generally have a shorter time lag between symptom onset and beginning of treatment and a higher chance of treatment success. In a retrospective study of 146 children with visceral leishmaniasis, AKI was diagnosed in 45.9% of cases according to pRIFLE criteria. Patients in the AKI group were significantly younger and had jaundice and secondary infections more often than non-AKI patients. The AKI group had significantly lower serum sodium, potassium, and albumin levels, elevated serum globulins and a more prolonged prothrombin time. The presence of secondary infections, reduced serum albumin levels, and increased serum globulin levels were considered risk factors for AKI in the studied patients. Death, mostly caused by septic shock, occurred in four patients (3.3%), younger than 1 year of age [128].

Pentavalent antimonial drugs are one of the therapeutic choices for treatment of leishmaniosis. These recognizably nephrotoxic class of medications is rapidly excreted in the urine after a parenteral dose. Although at low doses and, for a short period of time, the renal toxicity potential is low, their use should be avoided in patients with renal dysfunction. Causes of AKI after administration of pentavalent antimonial drugs include development of a urinary concentrating defect secondary to heavy metal renal toxicity, post-therapeutic massive deposition of immune complexes secondary to *Leishmania* destruction, and, rarely, interstitial nephritis [127].

Diagnosis

In cutaneous leishmaniosis, specimen collection should prioritize cutaneous and mucosal areas with macroscopic abnormalities; diagnosis can be confirmed in biopsy specimens by molecular and traditional methods.

Bone marrow aspiration is the preferred source for diagnosis of visceral leishmaniasis. Liver, enlarged lymph nodes, and whole blood (buffy coat) are other potential sources of tissue specimens.

Multiple diagnostic approaches are recommended to maximize the likelihood of a positive *Leishmania* result. Serologic testing (rK39) is recommended for persons with suspected visceral leishmaniosis in whom definitive diagnostic tests for the parasite, such as microscopic identification, culture, and molecular tests for parasite DNA, cannot be conducted or have negative results. The sensitivity and specificity of serologic tests depend on the assay and antigens used, as well as host factors. Serologic tests cannot be used to assess the response to treatment. Antileishmanial antibodies can be detected years after clinically successful therapy in some persons. Antileishmanial antibodies should not be performed as the sole diagnostic assay. Antibodies may be undetectable or present at low levels in persons with visceral leishmaniasis who are immunocompromised because of concurrent HIV/AIDS or other reasons (https://www.cdc.gov/parasites/leishmaniasis/resources/pdf/cdc_diagnosis_guide_leishmaniasis_2016.pdf, last access 10/24/20 and 1/2/2022).

Treatment

For detailed information on treatment of the various forms of leishmaniasis consultation of specialized texts is recommended [129, 130].

Trypanosomiasis

African Trypanosomiasis, also known as sleeping sickness, is a protozoal disease caused by motile hemoflagellates of the genus *Trypanosoma. Trypanosoma brucei rhodesiense* predominates in tropical east Africa while *Trypanosoma brucei gambiense* is found in tropical west and central Africa. The disease is transmitted by the bite of tsetse flies (genus *Glossina*). *T. b. rhodesiense* causes the more acute of the two forms, leading to death within 1 year if left untreated. The clinical picture associated with *T. b. gambiense* infection is usually characterized by several intermittent periods of clinical activity alternating with latent periods that persist for several years. The clinical stages of trypanosomiasis range from the trypanosomal chancre that appears at the site of inoculation to febrile attacks, diffuse intravascular coagulation (*T. b. rhodesiense*), lymphadenopathy (*T. b. gambiense*), and eventually progressive brain dysfunction leading to sleeping sickness, cachexia, and death. Although glomerulopathy associated with *T. brucei* infection has been described in several species, human glomerular disease is limited to an occasional report, same as the association of *Trypanosoma cruzi* infection (Chagas disease or American trypanosomiasis) with nephritis [114].

Trypanosomal nephritis presents as a spectrum from purely mesangioproliferative to membranoproliferative glomerulonephritis with the presence of IgM and IgG deposits and complement components in the mesangium and along the glomerular capillary walls, by immunofluorescence. On electron microscopy, large electron-dense deposits are observed in mesangial, subendothelial, and subepithelial areas. As with the other parasite-related glomerulopathies, an important pathogenic role for immune complexes is postulated in this disease [114]. The APOL1 G2 variant has been found to be strongly associated with five- to seven-fold reduced susceptibility to infection, evidenced by in vitro lysis of *T. b. rhodesiense* by plasma containing the *APOL1* G2 allele [130].

Trichenella

Trichinellosis is a parasitic infection caused by a nematode belonging to the genus *Trichinella*. Infection occurs by ingestion of infected pork, game, or horse meat, particularly in Europe and North America. The severity of the clinical course depends on parasitic factors such as the species involved, the number of living larvae ingested, and host factors such as sex, age, ethnic group, and immune status. Most infections are asymptomatic. The acute infection is two-phased; during the enteral phase, the parasite alters intestinal function, and the *parenteral phase* is characterized by an inflammatory and allergic response to muscle invasion by the larval parasites. The clinical presentation occurs predominantly by diarrhea, myositis, fever, prostration, periorbital edema, eosinophilia, and occasionally myocarditis, pneumonitis, or encephalitis. Infection severity depends on the number of ingested larvae and on treatment delay. Death may result from congestive heart failure due to myocarditis, encephalitis, pneumonitis, hypokalemia, or adrenal gland insufficiency but is presently rare because of improved treatment options [114].

Diagnosis is based on anamnestic criteria and the presence of hypereosinophilia, increased total IgE, and elevated muscle enzyme levels. Diagnostic confirmation relies on specific serological tests (enzyme-linked immunosorbent assay and immunoblot) and/or the finding of larvae in a muscle biopsy. Mebendazole, albendazole, or thiabendazole may be used for specific treatment [131].

The seemingly rare renal manifestations may present as glomerular disease, AKI, or transient urinary sediment findings. Literature reports describe severe fatal cases accompanied by acute tubular necrosis and myoglobinuria; while in milder cases, urinary sediment abnormalities have disappeared after effective infection control. A few cases of membranoproliferative glomerulonephritis associated with trichinosis have been described in which immune complexes were seen in glomeruli [114].

Echinococcosis (Hydatid Disease)

Hydatid disease is caused by *Echinococcus granulosus*, a tape worm whose eggs are passed in stool; sheep, cattle, goats, pigs, and camels are the common intermediate hosts. Infection is frequently transmitted to humans by dogs, who as humans are definite hosts for the disease. The disease's highest incidence occurs in countries where dogs are involved with sheep and cattle raising such as the Middle East, Australia, New Zealand, South Africa, South America, and the Mediterranean area. Worm embryos developed inside the eggs are liberated within the human gut, penetrate the intestinal mucosa, and reach the portal circulation. Most larvae are filtered out by the liver and lungs, but some organisms escape to the general circulation and affect other organs such as the kidneys (2%). The surviving larvae develop into hydatid cysts, which are unilocular, in contrast to those of *Echinococcus multilocularis*, which are multilocular. These cysts may be detected by accident or because of pain or accidental rupture, which causes allergic reactions and hypotension. The cysts develop more frequently in the liver (75%) and lungs (15%). Primary or sole involvement of kidneys is rare and accounts for 1–5% of all hydatid disease in humans [114].

Renal hydatid is frequently asymptomatic or presents with unspecified signs or symptom such as: palpable mass, flank pain, hematuria, malaise, and fever and, eventually, passing of daughter vesicles that can be identified as pearly structures in urine. Diagnosis of human cystic echinococcosis (CE) can be made by ultrasound or computed tomography. Laboratory confirmation relies on serological tests or, in patients with renal cysts, on the presence of daughter vesicles in the urine seen in 10% cases. Ultrasound imaging is the basis for the WHO/IWG-E – 2010 classification system for CE, which is used to guide treatment. Computed tomogram is considered superior to other imaging modalities in observing daughter cysts, minute calcifications, intracystic gas, and for preoperative anatomical localization. The treatment is surgical as simple aspiration is associated with exceedingly high recurrence rates (90%). Active and transitional lesions should be treated with a combination of surgical intervention and medical management due to high infectivity and risk of spread. Inactive lesions should be monitored. Surgical modality includes extraperitoneal and laparoscopic partial or total nephrectomy. The extraperitoneal approach is preferred as laparoscopic nephrectomy involves risk of cyst rupture, dissemination, and incomplete removal of the hydatid cyst. Total or partial nephrectomy is recommended with cyst rupture into the collecting system or outside.

Medical management is based on albendazole (10–15 mg/kg/day in two divided doses) or mebendazole (40 mg/kg/day in three divided doses). In inoperable cases, medical management should be associated with percutaneous aspiration injection and re-aspiration (PAIR) procedure in inoperable cases. Preoperative introduction of three cycles of mebendazole or albendazole, of 4 weeks duration 2 weeks apart, makes the cysts less tense preventing spillage and anaphylaxis. A 3-month post-nephrectomy course of albendazole may prevent recurrences [132].

There are a few descriptions of hydatid disease-associated glomerulopathy whose histopathology findings range from the membranous to the membranoproliferative glomerulonephritis. Glomerular involvement in *E. multilocularis* infection has not been described [114].

Fungal Infections

Systemic fungal infection affects children, infants, and neonates with an underlying compromised immune system. From a renal perspective, children at risk include those in pediatric or neonatal intensive care units, recipients of abdominal surgery or immunosuppressive medication, and those with autoimmune diseases [134]. Fungal diseases that specifically affect children on dialysis and following transplantation are addressed in respective chapters on dialysis and transplantation.

Candida is the commonest fungal species to cause blood stream infections. Among the species, *Candida albicans* is the most common causative fungus followed by *Candida* non-albicans [*C. parapsilosis*, *C. tropicalis*, *C. guilliermondii*, and *C. glabrata*]. Delayed central venous catheter removal, acute kidney injury, and dialysis are known to be associated with mortality due to systemic candidemia in children [135, 136].

The two broad groups of patients at risk to develop fungal infections relevant to pediatric nephrology practice are those admitted to PICU and preterm neonates in NICU [137]. Invasive fungal disease in the PICU setting is seen in high risk children with central venous catheters, urinary catheters, broad spectrum antibiotics, parenteral nutrition, and those exposed to systemic steroids or immunosuppressants. Invasive candidiasis, the commonest fungal infection in the PICU setting, is associated with central venous catheters and use of vancomycin for >3 days within the preceding 2 weeks [138]. The occurrence of invasive candidiasis in neonates is based on the birth weight with an incidence of 4–16% in extremely low birth weight neonates and 2–5% in very low birth weight neonates. The most common presentation is generalized sepsis with dissemination to kidneys in 5% neonates [137]. Specific renal-related risk factors for *Candida* infection include use of broad spectrum antibiotics, steroids, central venous catheters, parenteral nutrition, and following abdominal surgery [139]. Acute kidney injury is an independent risk factor for treatment failure [136].

Candida species have the ability to both colonize and invade the urinary tract. Candiduria can be an incidental finding in patients with sepsis but may not necessarily present with symptoms pertaining to the urinary tract. *Candida* pyelonephritis can present as febrile UTI or lower urinary tract infection. Rarer presentations include emphysematous pyelonephritis, perinephric abscess, fungal balls, and papillary necrosis. Figure 7 depicts the hyphae of candida in the kidney of an immunocompromised host. Asymptomatic candiduria needs to be addressed only in those who undergo a urological procedure, those with neutropenia and in low birth weight neonates. Renal candidiasis refers to a combination of candida in the urine and imaging evidence of renal parenchymal involvement or fungal balls in the urinary tract [140]. Obstructive renal candidiasis includes fungal balls causing urinary tract obstruction with associated oliguria/anuria, hypertension, flank mass, and renal dysfunction. Ultrasonography is useful in detecting fungal balls and features of urinary tract obstruction.

Diagnosis: Presence of yeast or hyphae under a direct microscopic examination with 10% KOH–calcofluor mount from a sterile site is significant. Urine culture with >10 [12] colony forming units per ml is diagnostic. More recently, non-culture methods available for diagnosis of invasive fungal infections include galactomannan for invasive aspergillosis; IA,1,3-β-D-glucan for aspergillosis, candidemia, and *Pneumocystis jiroveci* pneumonia; mannan antigen and anti-mannan antibody assays for candidiasis; latex agglutination and enzyme immune assay (EIA) for *Cryptococcus*. However, with limited data on the utility of these tools in children, no clear recommendation for their role in diagnosis can be laid out [134, 138].

Antifungal therapy: The “Clinical practice update of antifungal prophylaxis in immunocompromised children and the Clinical practice guideline for management of candidiasis” recommend antifungal prophylaxis in preterm neonates <1000 g body weight in those neonatal intensive care units with prevalence of candida infection above 5–10%. Prophylactic Fluconazole or oral

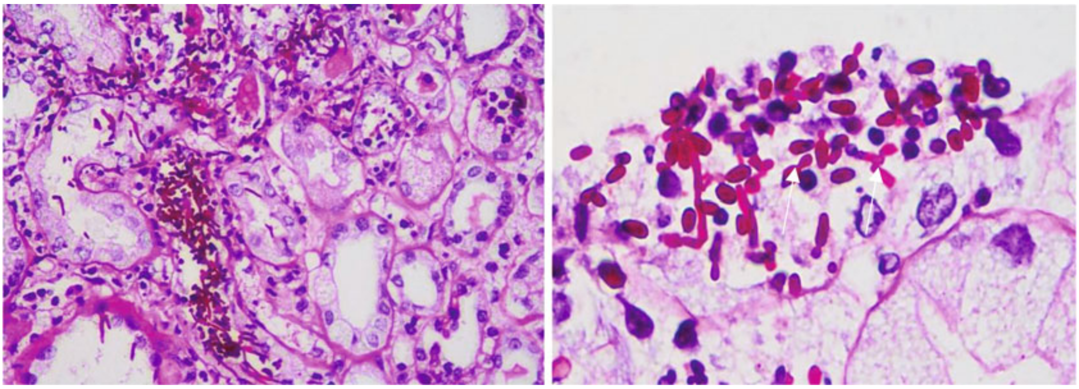

Fig. 7 (**a**) *Candida albicans* seen within the interstitium and tubular lumen PAS. (**b**) 40X magnification PAS image of budding and pseudohyphae forms (white arrows) of candida in an immunocompromised host. (Courtesy: Dr. Anila Abraham, Renopath, Centre for renal and urological pathology Pvt. Ltd. Chennai, India)

nystatin is indicated for 6 weeks. Neonatal candidiasis should be treated with Amphotericin B deoxycholate or Fluconazole for 2 weeks duration following the resolution of signs of candidemia. Lipid formulation of amphotericin B should be used with caution in the presence of urinary tract involvement and Echinocandins (Caspofungin and Micafungin) are indicated only for refractory infection. Similarly, in high risk pediatric patients with suspected invasive candidiasis, empiric treatment with echinocandins or fluconazole or lipid formulation of Amphotericin B are strongly recommended for 2 weeks duration [141, 142].

Others

Serious Forms of Urinary Tract Infection

The more serious forms of urinary tract infection include acute focal bacterial nephritis (AFBN), renal abscess, and pyonephrosis.

They are presently considered uncommon but are being increasingly reported due to improvements in the sensitivity of imaging techniques that enables their visualization. These clinical entities may be clinically similar to pyelonephritis, or may mimic abdominal inflammatory processes or tumors. The renal infectious process seems to be a continuum from pyelonephritis to abscess of ascending origin and xanthogranulomatous pyelonephritis; the different forms of clinical manifestations may be associated with urinary tract anatomy, renal vascular bed, bacterial properties, or host response to infection. Pyonephrosis should be included in the same inflammatory spectrum in obstructive or dilated collecting systems. The etiology of AFBN and of renal abscess of ascending origin is similar to that of other forms of UTI. Computed Tomography (CT) is considered to be the most sensitive and specific diagnostic method for imaging parenchymal and suppurative renal lesions. Magnetic resonance urography will possibly replace CT, as imaging methodology of choice, in the near future. Most of the described patients have pre-existing urological abnormalities, especially congenital urological malformations [143].

Acute Focal Bacterial Nephritis

Acute focal bacterial nephritis, previously known as acute lobar nephronia, is a localized, bacterial, inflammatory renal mass without liquefaction, which affects one or more renal lobules; it is regarded as an intermediary lesion between pyelonephritis and renal abscess. Characteristic CT findings are heterogeneous or wedge-shaped parenchyma with decreased enhancement. The absence of cortical rim sign can differentiate AFBN from renal infarction [142]. The administration of intravenous antimicrobial treatment until 2–3 days of fever abatement (at least 1 week) and a total treatment duration of at least 3 weeks are generally recommended in AFBN. High rates of renal scarring despite antibiotic treatment have been reported in children with AFBN [144].

Renal Abscess

Renal abscess is a focal, intrarenal (cortical) or perinephric, walled-off purulent parenchymal cavity. It is more frequently associated with an ascending urinary tract infection, although in approximately 30% of cases, it may be attributed to a hematogenous dissemination from other sites of infection such as wound infection, furuncles, or pulmonary infection. Perinephric abscesses are usually associated with a disruption of a corticomedullary intranephric renal abscess, recurrent pyelonephritis, xanthogranulomatous pyelonephritis, or an obstructing renal pelvic stone causing pyonephrosis. Gram-negative bacterial abscess commonly develops due to rupture of corticomedullary abscess while the staphylococcal infection develops due to rupture of a renal cortical abscess [145].

In children, fever and abdominal pain are the most common clinical symptoms. A previous genitourinary condition may be present in a third of patients. A positive history of previously diagnosed urinary tract infection may occur in third of the cases. Laboratory evaluation may reveal elevated C-reactive protein or erythrocyte sedimentation rate, in >80% of cases, and abnormal urinalysis in two-thirds of cases. *Escherichia coli* is the most frequently isolated microorganism and may occur in 50% cases while the isolation of

Staphylococcus aureus is less frequent ultrasound and computed tomography are the most used diagnostic imaging modalities. Treatment may be accomplished with intravenous antibiotics alone in most of the patients without the need for percutaneous drainage or surgery [146]. Antimicrobial treatment failure may be associated with large abscesses, obstructive uropathy, severe vesicoureteral reflux, diabetes, old age, and urosepsis with gas forming organisms. In the presence of a large abscess (in children >3 cm diameter) without clinical improvement after 48–72 h of adequate antibiotic therapy, a drainage procedure should be considered. The presence of obstructive uropathy indicates the need for prompt drainage, by percutaneous nephrostomy, followed by surgical correction of the primary obstructive uropathy, after the patient is stable and afebrile. Nephrectomy is indicated only for patients with diffuse damage of the renal parenchyma [145].

Xanthogranulomatous Pyelonephritis (XPN)

Xanthogranulomatous pyelonephritis (XPN) is a rare, atypical, and severe form of chronic inflammatory lesion of the kidney, whose pathomechanism is poorly understood. It is characterized by the destruction of the renal parenchyma and the presence of multinucleated giant cells and lipid-laden macrophages, as well as inflammatory infiltration and intensive renal fibrosis [146].

Pediatric XPN is rare and 60–75% of cases occur before 5 years of age. Its development is highly associated with congenital urinary tract malformations such as vesico–ureteral reflux, ureteropelvic junction obstruction, and bladder extrophy. Obstruction due to renal calculi has been reported in 38–83% of cases and 31–50% of the calculi have been of the staghorn variety. It may be related to inadequately treated chronic urinary infection by *Proteus* spp., *Escherichia coli, Pseudomonas* spp., and *Klebsiella* spp., lipid metabolism disorders, and altered immune response. Other possible etiological factors may include a lymphatic obstruction, malnutrition, arterial insufficiency, venous occlusion and hemorrhage, and necrosis of pericaliceal fat [146]. Two forms of XPN have been described: a diffuse form, which occurs in about 75% or 90% of cases, and a focal or pseudo-tumoral form, which although less frequent in general, is more commonly described in children (Fig. 8). The diffuse form is characterized by enlargement of the involved kidney with maintenance of its reniform shape, in association with nephrolithiasis, hydronephrosis, and loss and replacement of the normal corticomedullary junction by yellow masses extending to the renal pelvis and perirenal fat. The focal form is generally unilateral, confined to a renal segment or one pole of a duplex system, more frequently affecting the lower pole of the kidney. Bilateral XPN is extremely rare [146].

Clinical presentation is usually subacute or chronic with nonspecific symptoms such as loin or flank pain, intermittent fever associated with anorexia, asthenia, a palpable flank or abdominal mass, and malaise with weight loss. Acute fever and flank tenderness are most common in the diffuse forms. Urinary symptoms such as dysuria are uncommon. Laboratory results show a microcytic anemia, an elevated erythrocyte sedimentation rate, leukocytosis, thrombocytosis, and increased C-reactive protein in most patients. Urinalysis may show pyuria, hematuria, or proteinuria. Positive urine cultures are predictive for XPN but occur in only 70% of all patients. The most frequently isolated organisms are *Escherichia coli* and *Proteus mirabilis* [146].

Diagnostic imaging of diffuse XPN is based on computed tomography, which typically shows global renal enlargement with multiple low-attenuation rounded areas replacing the renal parenchyma, which represent dilated calyces or focal areas of destruction filled with pus or debris and have been named the “bear paw sign.” After intravenous contrast, an enhancement of these cavities’ walls, due to vascular granulation tissue, can be demonstrated The renal pelvis is usually contracted around a central calculus, although multiple and diffuse renal calculi may be found inside different calyces. Diagnosis of XPN may be inferred by the presence of a lipomatous component. Extrarenal disease may involve the perinephric and paranephric spaces, the posterior abdominal wall, or psoas

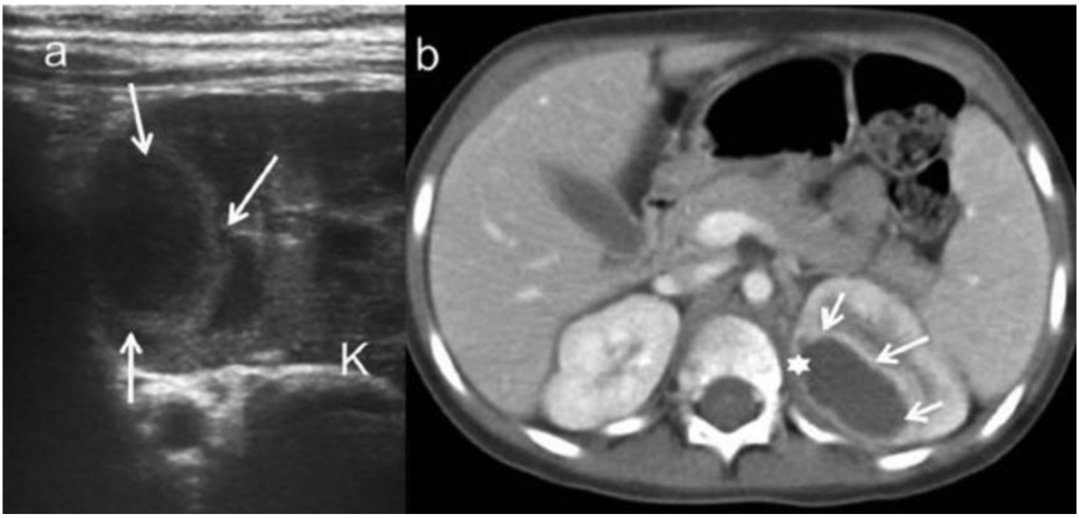

Fig. 8 Longitudinal sonographic image shows well-defined (white arrows) mass in upper pole of left kidney (K). (**b**) Contrast-enhanced nephrogenic phase transverse CT image shows hypodense lesion (asterisk) with rim enhancement (white arrows) [146]

muscle. Adenopathies are frequently present. MRI findings in diffuse XPN are variable, probably because the signal intensity of the solid component of XPN on T1-weighted images depends on the amount of xanthoma cells involved in the granulomatous process. Dimercaptosuccinic acid (DMSA) scintigraphy is helpful for the diagnosis of a non-functioning or very low renal functioning kidney. The definitive diagnosis is based on pathological findings after nephrectomy, which demonstrates areas of parenchymal destruction and necrosis as well as interstitial fibrosis and chronic inflammatory infiltrate composed of plasma cells, lymphocytes, and numerous lipid-laden macrophages. The absence of clear guidelines on the management of XPN obliges consideration of conservative and surgical treatments for each individual case [146].

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Viral Infections and the Kidney

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Patricio E. Ray, Asha Moudgil, and Aditi Sinha

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Abstract

Viral infections are associated with a number of renal diseases in children resulting in significant morbidity and mortality worldwide. The role of viral infections in the pathogenesis of kidney disease has been largely based on clinical and histologic kidney findings in temporal association with a systemic viral infection and the demonstration of kidney cells infected with viral particles. Viral infections associated with productive viral replication in the kidney of immunocompromised hosts are of particular concern for children. Here, we will discuss the epidemiology, pathogenesis, outcomes, and treatment of the most relevant kidney diseases associated with viral infections in children, excluding those occurring in kidney transplant recipients, which are discussed elsewhere in this textbook (see chap. 84, Renal transplantation: infectious complications). Well-defined associations between a viral infection and kidney disease have been made for the hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV-1), hantaviruses, and the new coronavirus SARS-CoV-2. In children with immunodeficiency states, herpes viruses, polyomavirus, and adenovirus have been recognized to induce kidney diseases. The roles of other viruses in the pathogenesis of kidney diseases are not clearly defined and remain speculative. With increasing application of molecular techniques, the understanding of the role of viruses in the pathogenesis of kidney diseases is expected to increase. In addition, more specific antiviral treatments and vaccines are currently under development and are expected to improve the outcome of viral-mediated kidney diseases in children.

Keywords

Childhood viral kidney diseases · Polyoma virus · Adenovirus · Hepatitis B and C viruses · HIV-nephropathies · Parvovirus B19 · SARS-CoV2 · Viral hemorrhagic fevers

Viruses

Viruses are the smallest and simplest living organisms ranging in size from 20 nm to 300 nm. They can multiply only inside living cells and require a host cell to survive. A virion usually consists of a nucleic acid core (DNA or RNA, but never both), an outer protein coating or capsid, and sometimes and outer envelope made of protein and phospholipid membranes derived at least partially from the host cell. Viruses can be classified based on their shape in four groups: filamentous, isometric or icosahedral, enveloped, and with head and tail. In addition, they have been classified into seven types based on their unique replication strategy [1]. In this chapter, we will discuss several types of viruses that cause renal diseases. These include Class I or double-stranded DNA viruses (herpes viruses, BKV, adenovirus), Class II or single-stranded DNA viruses (Parvovirus B 19), and Class IV or single-stranded positive sense RNA viruses (Hepatitis C, SARC-CoV-2); positive-sense viral RNA, like mRNA, can be directly accessed by host ribosomes and immediately translated into proteins. Other viruses discussed include Class IV, single-stranded negative sense RNA viruses (Ebola, Hantavirus, other viruses that cause hemorrhagic fevers). In these latter viruses, the negative-sense viral RNA cannot be directly accessed by host ribosomes to form proteins, and needs to be transcribed into positive-sense RNA. Other viruses discussed are Class VI or positive sense single-stranded RNA viruses that replicate through DNA intermediates (HIV-1 and other retroviruses). Finally, class VII or double-stranded DNA viruses that replicate through a single-stranded RNA intermediate (HBV) will be discussed.

The first step in viral replication is the attachment of the virus to specific host cell surface receptors. This step is followed by the viral entry step into the cells, usually involving receptor mediated endocytosis. Once inside the cells, the virus is uncoated to release its viral nucleic acids. Thereafter, viral DNA or RNA replication occurs, when the machinery to produce cellular protein of the host cells is used to produce viral proteins. Crossing the nuclear membrane is a critical barrier that some viruses must face to replicate. Viruses that cannot cross the nuclear membrane (e.g., parvovirus B19) can replicate only in actively dividing cells, when the nuclear membrane breaks down during mitosis. Finally, viral proteins are assembled in the nucleus or cytoplasm, depending on the type of DNA or RNA virus, and packaged into virions before they are released from the cells by either budding or lysis. In budding, the mechanism used by HIV-1, the virus incorporates some of the host's cell membrane as it leaves and preserves the cell.

Viruses are continuously evolving and have developed several mechanisms to survive in host cells [2]. They can undergo mutations in different viral sites to avoid recognition by the host's immune system. In order to replicate, the RNA viruses must encode RNA polymerase enzymes that are needed to convert RNA into DNA which are not present in the host's cells. These RNA polymerases make more copying errors than the DNA polymerases encoded by the DNA viruses, leading to frequent mistakes during transcription. Therefore, RNA viruses undergo more frequent mutations and can change and adapt more rapidly to survive in their host cells. Viruses, may also become latent or silent inside the cells, or produce viral proteins capable of impairing the host's immune response. The cytomegalovirus (CMV) modulates the expression of MHCI and II cell surface molecules, and the HCV virus blocks a key innate immune response in the interferon pathway that protects against viral infections. Finally, other viruses modify the function of cytokines and chemokines by producing viral homologues of these key host proteins.

Viral Infections and Kidney Diseases

Several viruses discussed above can induce kidney injury directly or indirectly by affecting specific glomerular and tubulointerstitial compartments. Table 1 shows that different viruses can cause either similar or different kidney pathologic phenotypes [3]. However, establishing causality of the kidney disease with a specific virus requires definitive diagnostic evidence of viral infection along with renal histological features consistent with the damage caused by the virus in infected cells. Infection of kidney cells can be documented by detecting viral antigens by immunohistochemistry, viral DNA or RNA by the polymerase chain reaction (PCR), and/or viral particles by electron microscopy or cultures. A common virus capable of infecting tubular epithelial cells is BKV, which causes a tubulo-interstitial nephritis (TIN) mimicking an acute rejection episode in renal transplant recipients [4]. Latent BKV infection is widespread in the general population, and reactivation of latent BKV in immunosuppressed patients can lead to TIN in these patients. Adenovirus infections can cause severe necrotizing granulomatous TIN with granulomas surrounding severely injured and virally infected distal tubules characteristic of this infection. CMV can rarely cause patchy TIN with lymphocytes, plasma cells, and macrophages. In CMV-induced tubulitis, epithelial cells, and endothelial cells can show the characteristic intranuclear glassy-appearing basophilic inclusions with a surrounding halo ("owl's eye-type" inclusion), and a marked increase in size (cytomegaly). Specific diagnosis is confirmed by immunohistochemical staining for CMV.

Viruses can also cause renal injury by increasing the recruitment of inflammatory cells, by facilitating the formation and deposition of immune complexes containing viral antigens, and/or by

Table 1 *Renal histological phenotypes* associated with different viral infections

Pathology	Viruses
Minimal change	ADV, HSV, VZV, CMV, EBV, PV, influenza, parainfluenza, HAV, HIV, PV
FSGS	ADV, HSV, VZV, CMV, EBV, PV, influenza, parainfluenza, HAV, HIV, PV
CG	HIV, PVB19, COVID-19
ICGN	ADV, VZV, mumps, CMV, EBV, influenza, HAV, HBV, HIV, PV, dengue, Coxsackie virus, ECHO virus
MesPGN	HSV, VZV, CMV, measles, mumps, rubella, HBV, HIV, PV, Togavirus
HSP and IGAN	HSV, VZV, measles, mumps, rubella, CMV, HBV, HIV, PV
MPGN	PV, influenza, Hepatitis A, B and C
Membranous nephropathy	EBV, CMV, HBV
Thrombotic microangiopathy	ADV, VZV, EBV, influenza, Coxsackie
Renal vasculitis	EBV, PV, influenza, Togavirus, HBV
TIN	CMV, BKV, ADV, EBV
Hemorrhagic cystitis, UVJO	BKV, ADV

Abbreviations. *GC* Collapsing glomerulopathy, *FSGS* Focal and segmental glomerulosclerosis, *ICGN* immune complex glomerulonephritis, *MesPGN* Mesangial proliferative glomerulonephritis, *HSP* Henoch Schonlein purpura, *IGAN* IGA nephropathy, *MPGN* Membranoproliferative glomerulonephritis, *TIN* Tubulointerstitial nephritis, *UVJO* ureterovesical junction obstruction

Viruses. Adenovirus (ADV), Herpes simplex virus (HSV), Varicella Zoster Virus (VZV), Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Parvovirus (PV), Hepatitis A virus (HAV), Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV)

Table adapted from Wenderfer S.E. Viral-associated glomerulopathies in children [3]

inducing autoantibodies against intrinsic renal antigens due to molecular mimicry [5, 6]. Viral particles may become trapped in the glomerular filtration membrane, leading to in situ immune-complex (IC) formation, or act as an antigenic stimulus to induce autoimmunity against cross-reactive glomerular cell epitopes [7]. In addition, direct infection of renal cells may either modify a tissue antigen or a cellular immune response to evoke autoimmunity [8]. Endothelial cells are especially susceptible to the pathogenic effects of several viruses, since they carry a vast array of molecules that modulate the adhesion of leukocytes and platelets, and/or cause complement activation. Maintaining intact endothelium is essential for the normal function of the glomerular filtration barrier, and to prevent thrombotic microangiopathic lesions. Viral pathogens frequently induce the activation of endothelial cells through cytokines and leukocytes, and may disrupt the integrity of renal vessels causing vascular leakage or bleeding. Endothelial injury is implicated in the pathogenesis of viral hemorrhagic diseases, including dengue, hantaviruses, and arenaviruses [9, 10]. Activation of complement and coagulation cascades is associated with clinical symptoms consistent with hemolytic uremic syndrome (HUS) in patients with COVID19 [11].

Specific Viral Kidney Diseases

Parvovirus B19 (PVB19)

Epidemiology

PVB19 is a small, non-enveloped, single-stranded DNA virus with humans being the only host. Infections with PVB19 are very common, occur worldwide, and can cause fifth disease in children. Viral acquisition often occurs during childhood although it does occur at lower rate throughout adulthood; 70–85% adults show serologic evidence of past infection. Infectivity varies by season and is more common in winter and spring. Transmission of infection usually occurs by inhalation of virus in aerosol droplets. PVB19 can be transmitted vertically from mother to fetus and less commonly through transfusion of blood products, bone marrow, and solid-organ transplants [12].

Pathology

Various renal pathologies described in association with PVB19 include acute proliferative glomerulonephritis, collapsing glomerulopathy (CG)

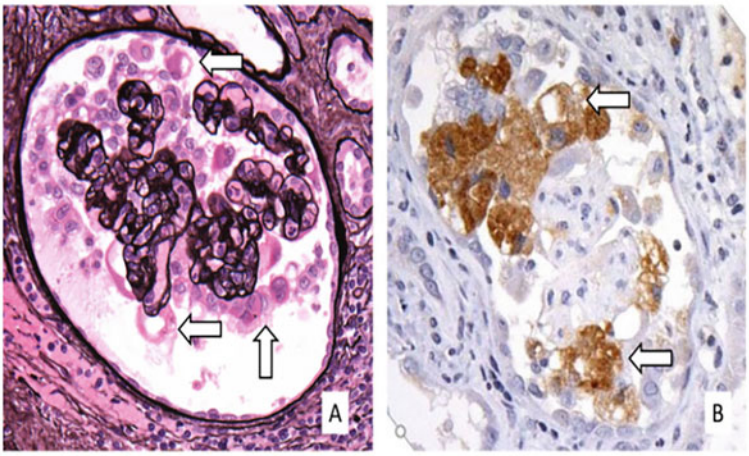

Fig. 1 Parvovirus B19 infection. (A) Collapsing glomerulopathy with hypertrophic and hyperplastic epithelial cells (arrows) and capillary luminal obliteration due to collapse (Jones Silver stain x160 magnification). (B) Immunohistochemistry stain for parvovirus B19 showing infected hyperplastic and hypertrophic glomerular epithelial cells (arrows) (x160 magnification). Courtesy: Dr. Cynthia Nast, Cedars-Sinai Medical Center, LA, CA

(Fig. 1A), focal and segmental glomerulosclerosis (FSGS), thrombotic microangiopathy (TMA), and necrotizing renal vasculitis in association with systemic vasculitis including Henoch Schonlein purpura (HSP), polyarteritis nodosa (PN) and Wegener's granulomatosis [13–15]. CG has been also described in renal transplant recipients infected with PVB19 [16]. Viral genome components have been detected in renal biopsies by PCR, and B19 antigen has been observed in the glomeruli by immunohistochemistry [17].

Pathogenesis

PVB19 has been implicated in the pathogenesis of several renal lesions, but its role in the pathogenesis is associative and speculative rather than causative. The role of PVB19 in CG has been suggested based on the higher prevalence of PVB19 DNA by PCR and in situ hybridization in renal biopsies of patients with CG as compared to other nephropathies (Fig. 1) [17]. However, it is unclear if the presence of the virus is pathogenic. PVB19 DNA has been localized to the glomerular podocytes and parietal epithelial cells, both of which have been implicated in the pathogenesis of CG (Fig. 1B). NS1, a viral protein of PVB19, has been shown to be cytotoxic in some non-renal cells [18]. It is not known whether this protein is injurious to kidney cells, but it cannot be excluded as a possible mechanism of glomerular injury.

Presentation

Acute onset of glomerulonephritis (hematuria, proteinuria, or hypertension) or rarely nephrotic syndrome (NS) after onset of PVB19 infection has been described in normal hosts, those with sickle cell disease, and rarely in renal transplant recipients [11–15].

Management

Treatment is mostly supportive but intravenous immunoglobulin (IVIG) therapy may be helpful [16].

Herpes Viruses

The structure of herpes viruses consists of a double-stranded linear DNA genome enclosed within an icosahedral protein capsid, and surrounded by a lipid bilayer envelope membrane. Eight herpes viruses infect mainly humans. These include human herpes simplex virus type 1 (oral herpes);

herpes virus simplex virus type 2 (genital herpes), varicella zoster virus (VZV), CMV, Epstein Barr virus (EBV), human herpes virus (HHV) 6 (cause of sixth disease), HHV 7, and HHV 8 (Kaposi's sarcoma herpesvirus). All these viruses can be reactivated in immunocompromised patients including those with kidney transplants. In addition, cases of IgA nephropathy, membranoproliferative glomerulonephritis (MPGN), and membranous nephropathy (MN) have been associated with herpes viruses, although little evidence has supported causality, with some exceptions. For example, while the association of varicella with nephritis has been known for decades, varicella only rarely causes fulminant renal disease in immunocompromised patients. Conversely, infectious mononucleosis, induced by the Epstein Barr virus, can cause transient hematuria and mild proteinuria for a few weeks [19]. More severe renal involvement with EBV is unusual, except for severe cases with hepatic failure, thrombocytopenia, and/or encephalitis, which can be associated with interstitial nephritis and acute kidney injury (AKI).

Cytomegalovirus (CMV)

Epidemiology

CMV is a member of the herpesvirus family. Prevalence of seropositivity increases with age and ~ 50% of people become seropositive by mid-adulthood in the United States [20]. Outside of the Unites States, the CMV seroprevalence rates run between 30% and 97% [21]. Virus transmission can occur by exposure to infected body fluids such as human milk, saliva, urine, blood and by organ transplantation. Primary CMV infection may be asymptomatic or may manifest as a self-limited febrile illness in immunocompetent individuals. After primary infection, CMV persists as a latent virus, which serves as reservoir for reactivation and transmission to susceptible individuals, such as infants and solid organ transplant recipients.

Pathology

CMV infection can cause both tubular and glomerular lesions. TIN can occur in native kidneys in congenital CMV infection and in renal allografts (Fig. 2A, B). Glomerular lesions associated with CMV infection include acute proliferative GN or severe glomerulonephritis with crescent formation [24]. Rarely, CMV can also cause CG in grafts from donors with APOL1 mutations [4, 22]. CMV is also known to cause chronic vascular injury. Cytopathic changes caused by CMV are characterized by intranuclear and cytoplasmic viral inclusions. Often, nuclei show an "owl's eye" appearance or sometimes appear smudgy. CMV infection can be diagnosed by immunocytochemical staining to detect CMV antigens. Electron microscopy can demonstrate viral particles measuring about 150 nm, often having a central electron-dense core and envelope in tubular cells and appearing as viral immune deposits in glomerular cells [23].

Pathogenesis

CMV infection can directly cause damage to tubular epithelial cells, glomerular and vascular endothelial cells, and mononuclear cells. Immune-complex GN has been documented with mesangial deposits of IgG, IgA, C3, and CMV antigens within glomeruli.

Presentation

Infection with CMV is usually asymptomatic in most patients except in those infected congenitally or that are immunocompromised. CMV positive donor/CMV negative transplant recipients have the highest reported rates of both CMV infection and CMV disease in the first 3 months after organ transplantation, although both can occur later [18]. Patients who are CMV seropositive at the time of transplantation are also at risk of reactivation and superinfection. The laboratory methods to identify a CMV infection include antigen detection, quantitative nucleic acid amplification testing (qNAT) by PCR, histopathology, and culture [18]. qNAT testing is the preferred method for routine CMV monitoring and diagnosis after transplantation.

Management

Antiviral prophylaxis and preemptive therapy are the mainstay therapies for CMV prevention.

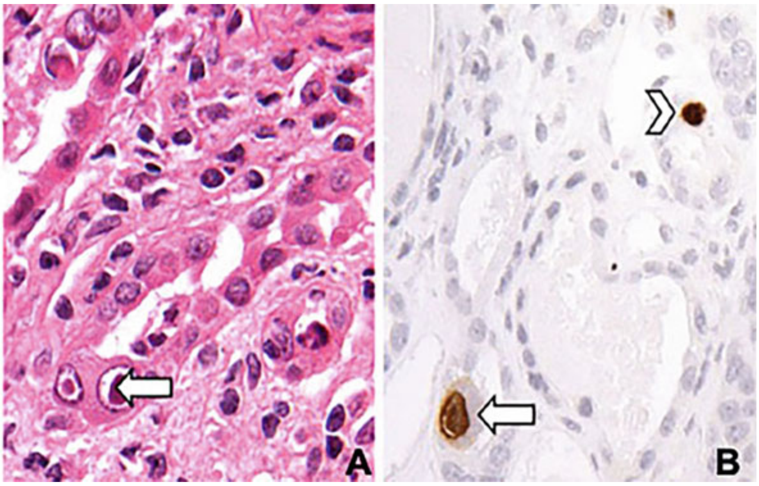

Fig. 2 CMV infection. (A) Enlarged tubular cell nucleus with a large purple intranuclear inclusion (arrow) with a smaller inclusion in the cell to the left (cytopathic change). Note the sloughed degenerated cells in the tubular lumens (H&E x240 magnification). (B) Immunohistochemistry stain for CMV showing an infected tubular cell nucleus (arrow). An infected tubular cell that has sloughed into a tubular lumen is also present (chevron) (x240 magnification). Courtesy: Dr. Cynthia Nast, Cedars-Sinai Medical Center, LA, CA

Antiviral agents shown to be effective against CMV include ganciclovir, valganciclovir, foscarnet, and cidofovir. Oral valganciclovir is recommended for CMV prophylaxis in the post-transplant period. Duration of chemoprophylaxis is dependent on the serostatus of the donor and recipient. Intravenous ganciclovir or oral valganciclovir can be used to treat CMV disease depending upon the severity of disease [18]. Major limitations of ganciclovir therapy include bone marrow toxicity and renal tubular dysfunction. Dosage adjustment is needed in the presence of renal dysfunction. Use of other antiviral agents such as foscarnet and cidofovir is often limited because of nephrotoxicity. The role of CMV immunoglobulin therapy in reducing severe CMV-associated disease after solid organ transplantation remains unclear [18].

Adenovirus

Epidemiology

Adenoviruses (ADV) are medium sized (90–100 nm), non-enveloped icosahedral viruses with double-stranded DNA. ADV infections are endemic in pediatric populations and people living in close quarters (college students and military recruits). The true incidence of ADV infection is not clear. ADV infection is asymptomatic in the normal host, but may cause hemorrhagic cystitis and or TIN in immunocompromised children, including bone marrow transplant recipients, those receiving intensive chemotherapy or rarely those that are kidney transplant recipients. Severity of disease and symptoms vary and are dependent on the serological subtypes of ADV, patient age, type of underlying immunosuppression and type of tissue/organ transplant. ADV infections are most often caused by subgroup B, serotypes 7, 11, 34, and 35. Serotypes 11, 34, and 35 are associated with hemorrhagic cystitis and severe interstitial nephritis. Recombinant adenoviral vectors are also used as a gene transfer tool. These vectors are generated by replacing the E1 gene, which is essential for viral replication, with the transgene of interest and an enhancer promoter element. Human kidney epithelial, mesangial, and endothelial cells can all be transduced successfully with these vectors [24], further supporting the notion that ADV can directly infect intrinsic renal cells. Modified human and monkey adenoviral vectors are currently being used to generate vaccines against COVID-19.

Pathology

Most ADV require the Coxsackie-virus B adenovirus receptor (CAR) to infect renal epithelial cells. ADV bind to this primary receptor and enter cells via endocytosis or micropinocytosis. Renal manifestations include features of TIN, with severe cases showing extensive tubular necrosis and destruction and rupture of tubular basement membranes and focal interstitial hemorrhage. The interstitial inflammatory response may sometimes be nodular and/or granulomatous [25].

Presentation

ADV infections manifest variably, ranging from asymptomatic infections to severe, prolonged, disseminated disease with significant morbidity, mortality and/or allograft loss. ADV are a major cause of hemorrhagic cystitis, which is more common in boys than girls with longer periods of microscopic hematuria, dysuria, and frequency in the former [19]. Renal manifestations of ADV infections include hematuria, proteinuria, and increases in serum creatinine levels. Diagnosis can be confirmed by immunohistochemistry or by electron microscopy. Additionally, ADV can be detected in the blood and urine by qNAT testing or by culture.

Management

Reduction of immunosuppression should be the initial approach in the treatment of adenovirus infections in transplant patients. Cidofovir should be considered as the next line treatment for ADV-associated disease. Patients given cidofovir should be well hydrated and probenecid can be administered to minimize the risk of nephrotoxicity. The ideal dose and frequency of cidofovir administration is not known, although dosing adjustments are needed for renal insufficiency [26].

BK-Virus (BKV)

Epidemiology

BKV is a non-enveloped, icosahedral encapsulated circle double stranded DNA virus. BKV infection is widespread and is typically acquired in childhood [27]. Approximately 80% of the population is seropositive for BKV by adulthood. Following primary infection, BKV frequently establishes latent infection in renal tubular cells and urinary tract epithelia [28]. Viral replication can occur under immunosuppression. BKV infection causes kidney disease only in immunocompromised hosts, most often in renal allograft recipients and rarely in other organ transplants. The estimated prevalence of BK-associated nephropathy (BKN) ranges from 1–5.5% [29]. Risk factors for infection include intense immunosuppression, higher degree of HLA mismatch and ureteral stent placement.

Pathology

The re-activation of silent BKV infections in immunosuppressed patients plays a critical role in the pathogenesis of BKV viruses. The specific mechanisms of viral activation remain unknown; most believe that tubular cells infected with BKV elicit an inflammatory reaction that is mediated by lymphocytes and monocytes/macrophages and that is centered around tubules which may develop large intranuclear inclusions with a cytopathic effect. BKV causes direct damage to renal epithelial and urothelial cells. Morphologic features of BKN include characteristic findings of TIN with mixed inflammatory infiltrates (lymphocytes, monocytes, and plasma cells) and tubular epithelial cells with viral intranuclear inclusions (Fig. 3A). Presence of large numbers of plasma cells within inflammatory infiltrates is consistent with an active polyoma virus infection. In addition, the humoral response can lead to immune complex deposition in tubular basement membranes. The diagnosis of BKN can be confirmed by immunohistochemistry (Fig. 3B), in situ hybridization or electron microscopy showing characteristic viral particles of about 30–40 nm in diameter.

Presentation

BKV in immunocompetent hosts are usually asymptomatic. Sometimes, healthy children can experience mild fever, malaise, vomiting, respiratory illness, pericarditis, and transient hepatic dysfunction. Most often, BKV infection is asymptomatic in renal transplant recipients and is

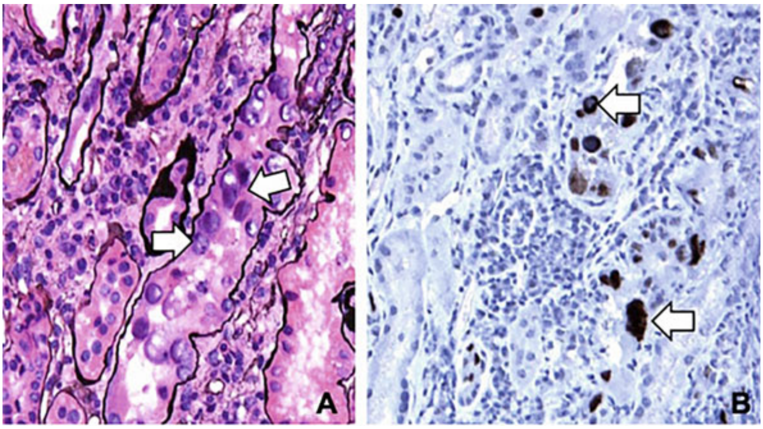

Fig. 3 Polyoma (BK) virus nephropathy. (A) Tubular cell nuclei (arrows) showing "cytopathic" change with enlarged nuclei containing viral inclusions and tubular cell degeneration with sloughing into the lumen (Jones silver x160 magnification). (B) Immunohistochemistry for polyoma virus (SV40 antigen) showing positive staining within enlarged tubular cell nuclei (arrows) (x160 magnification). Courtesy: Dr. Cynthia Nast, Cedars-Sinai Medical Center, LA, CA

discovered during routine monitoring by qNAT testing which has become the standard of care and recommended by the Kidney Disease: Improving Global Outcomes (KDIGO) group. BK nephropathy usually presents as renal allograft dysfunction and rarely with proteinuria and hematuria. BKV infection can rarely cause ureteral stenosis and hydronephrosis [30].

Management

Reducing immunosuppression may be helpful in clearing the infection. Role of other therapies such as leflunomide, ciprofloxacin, cidofovir, and IVIG is less well established [31].

Coronavirus

Coronaviruses are enveloped, single-stranded RNA viruses. Most coronaviruses cause mild respiratory infections without renal involvement [32]. However, in the last 20 years, three potentially lethal coronaviruses had emerged that induce a severe acute respiratory syndrome (SARS) with multiorgan involvement and kidney complications. The first potentially lethal coronavirus emerged in South China in 2002, and was named SARS-coronavirus (SARS-CoV) [33]. Patients developed severe pneumonia with diffuse alveolar damage and hypoxemia. Kidney complications were seen in the most severe cases with multiorgan involvement [34]. SARS-CoV enters cells via angiotensin converting enzyme 2 (ACE2) which is expressed in several renal cell types [35], thus explaining the renal tropism of this virus. The second virus emerged as an outbreak in the Middle East in 2012, and was named Middle East Respiratory Syndrome-coronavirus (MERS-CoV). This virus causes similar symptoms than SARS-CoV but resulted in higher mortality rates (~40%) and more kidney complications. The renal complications were attributed to both the multisystemic involvement and renal tropism of the virus [36]. The third potentially lethal coronavirus, SARS-CoV2, emerged in Wuhan, China in December 2019, and caused a major global pandemic of a severe respiratory illness named COVID-19 (coronavirus disease 2019).

COVID-19

Epidemiology

SARS-CoV2 is an enveloped positive sense-single stranded RNA virus that causes COVID-19. The incubation period is up to 14 days, with most infected individuals having symptoms around 4–5 days after exposure; however, case reports with longer incubation periods (up to 27 days) have been reported [37]. SARS-CoV2 tends to cause more serious disease in older adults and those with underlying co-morbidities that include obesity, hypertension, diabetes mellitus, and smoking. Children are less often and less seriously affected [38].

Pathology

Virus-mediated injury to renal tubules, podocytes, and endothelial cells has been described in autopsy studies. Viral particles have been detected in renal tubular epithelial cells and podocytes by electron microscopy and SARS-CoV-2 nuclear protein has been observed in proximal tubular cells by immunofluorescence. Acute tubular injury and collapsing glomerulopathy have been described in association with COVID-19 [39, 40]. However, the pathophysiology of the kidney injury in patients with COVID-19 has not been fully elucidated [41]. In particular, it is unclear whether kidney epithelial cells are exposed to a high viral load (as occures IN lung and nasal epithelium), since the virus is rarely detected in the blood and urine. Furthermore, the development of AKI appears to be multifactorial, similar to the pathophysiology of AKI in patients with sepsis or those who become critically ill when infected with viruses that induce hemorrhagic fevers [41].

Pathogenesis

Kidney cells appear to be vulnerable to SARS-CoV-2 infection because they express angiotensin converting enzyme 2 (ACE-2) and transmembrane serine protease 2 (TMPRSS2), two molecules required for the attachment and entry of SARS-CoV-2 into cells. These molecules are expressed mainly by renal proximal tubular cells and to a lesser extent by intercalated and principal cells of the collecting duct, distal tubular cells, parietal epithelial cells, and infiltrating immune cells. SARS-CoV-2 can cause direct damage to podocytes explaining occurrence of proteinuria and hematuria. AKI can occur secondary to the cytokines released by activated macrophages that infiltrate the kidney, activation of the angiotensin II pathway, dysregulation of complement, hypercoagulation, and microangiopathy [42]. In conclusion, the renal damage observed in patients with COVID-19 appears to be the result of multiple mechanisms [41], including direct cytopathic effects, immune-mediated damage, rhabdomyolysis, impairment of renal microcirculation, and disseminated intravascular coagulation [37]. Cases of COVID-19 associated AKI progressing chronic kidney disease (CKD) have been reported.

Presentation

Clinical manifestation of infection in children include no symptoms to mild symptoms including fever, dry cough, fatigue, and nasal congestion. Some children may have gastrointestinal symptoms including abdominal discomfort, nausea, vomiting, abdominal pain, and diarrhea. Most pediatric patients recover within 1–2 weeks. Few progress to having lower respiratory infections. Hypoxemic respiratory failure needing ventilator support and hypotension are the most common reasons for intensive care unit admission for patients with severe COVID-19. Renal manifestations, if present, are usually seen in patients with severe COVID-19 infections. Proteinuria and hematuria are common signs of renal involvement among hospitalized patients. AKI can occur in seriously ill patients and appears to result from a complex process that includes traditional risk factors such as hypotension and hypoperfusion of the kidneys and COVID-19 specific factors. Renal involvement in COVID-19 is associated with increased mortality [43]. Diagnosis is made by a real time polymerase chain reaction (RT-PCR) test to detect SARS-CoV-2 nucleic acid in nasopharyngeal swabs.

Management

Children with SARS-CoV-2 infection with mild symptoms usually only require antipyretic therapy. In cases of dehydration, vomiting, or diarrhea, the use of ibuprofen should be avoided due to the risk of AKI. As only a few cases of severe COVID-19 in children have been reported including multisystem inflammatory syndromes, limited evidence of good quality is available to recommend treatment for these patients. Some evidence suggests that intravenous corticosteroids and IVIG may be beneficial in severe cases [44]. The value of other treatments, including hydroxychloroquine, remdesivir, ivermectin, nebulized interferon alpha-2b, and oral lopinavir/ritonavir, is still unclear at the present time, and should be used only in the context of clinical trials [44]. Currently, no solid data supports that angiotensin converting enzyme inhibitors or angiotensin receptors blockers increase the susceptibility of people to a SARS-CoV-2 infection; thus, these drugs should not be discontinued in infected patients. Continuous renal replacement therapy can be used in critically ill children with AKI. Hypercoagulability leading to frequent filter clotting may occur in these children. Increased doses of unfractionated heparin, combination of heparin and regional citrate anticoagulation, or combination of prostacyclin and heparin are potential treatments for these issues. Acute peritoneal dialysis or intermittent hemodialysis can be used in patients who are hemodynamically stable [45].

Hepatitis B Virus (HBV)

Epidemiology

HBV is a partially double-stranded DNA virus comprised of an outer surface envelope (HBsAg) and an inner nucleocapsid containing the hepatitis B core antigen (HBcAg) and viral DNA. The HBcAg releases the hepatitis B antigen antigen into the circulation, and this antigen indicates the presence of infectious viral particles. Chronic HBV infection, defined as persistent circulating hepatitis B surface antigen (HBsAg), affects 5–6% of the population globally [46]. Its prevalence varies from 0.1% in the United States to 2%–7% in south Asia and 20%–30% in Pacific Islands [46]. Infants infected at birth or immunosuppressed children are most likely to be infected chronically. While only 3%–5% of viremic patients develop glomerular disease, the infection accounts for 25% of patients with specific glomerulopathies in endemic regions [47, 48]. Occult HBV infection (viral nucleic acid in liver, kidney, peripheral blood mononuclear cells or ultracentrifuged plasma, but not amplified in serum by conventional PCR) also occurs, particularly with membranous and/or IgA nephropathy [47]. Co-infection with HCV or HIV is observed in 20%–30% and 5%–10% cases, respectively, and can alter glomerular pathology and plan of management [47]. Overall, the incidence of glomerular disease attributed to HBV mirrors geographic variations in its prevalence [49, 50], and is declining in regions with universal HBV vaccination in childhood [51].

Pathology

The most common pattern of glomerular injury associated with chronic HBV viremia is MN, particularly in children [47, 48] (Fig. 4A). These patients have deposits of IgG, C3, IgM, and IgA, in subepithelial, subendothelial, and mesangial cells. HBV is also associated with polyarteritis nodosa (PAN), seen more frequently in cases of subacute or chronic viremia (weeks to years). Diffuse proliferative GN has been associated with acute viremia (days to weeks). People with chronic viremia can also develop immune-complex MPGN with or without mixed cryoglobulinemia, IgA nephropathy, focal segmental glomerulosclerosis (FSGS), mesangioproliferative GN, amyloidosis, antineutrophil cytoplasmic antigen (ANCA) negative necrotizing small-and medium-vessel vasculitis, and fibrillar or immunotactoid glomerulopathies.

Pathogenesis

Active viremia is required to cause kidney disease, either through deposition of immune complexes and/or direct viral infection [52]. The histological pattern of injury reflects the antigen in the immune complexes. Subepithelial deposits, mimicking typical MN, usually contain cationic HBeAg-IgG that readily traverses the glomerular filtration barrier as HBeAg is of relatively small size (~17 kD) [47, 48]. In contrast, anionic IgG-HBsAg complexes are larger (HBsAg is ~35–50 kD) and therefore restricted to the subendothelial space, leading to MPGN in glomeruli and PAN in medium-sized vessels [53]. HBsAg is sometimes associated with mixed cryoglobulinemia type III and direct tissue infection may cause IgA nephropathy or FSGS [47, 48].

Patients with HBV-associated MN almost always have circulating HBeAg [47, 52]. Viral genotype A and specific acquired missense mutations in the HBV X gene predispose to developing

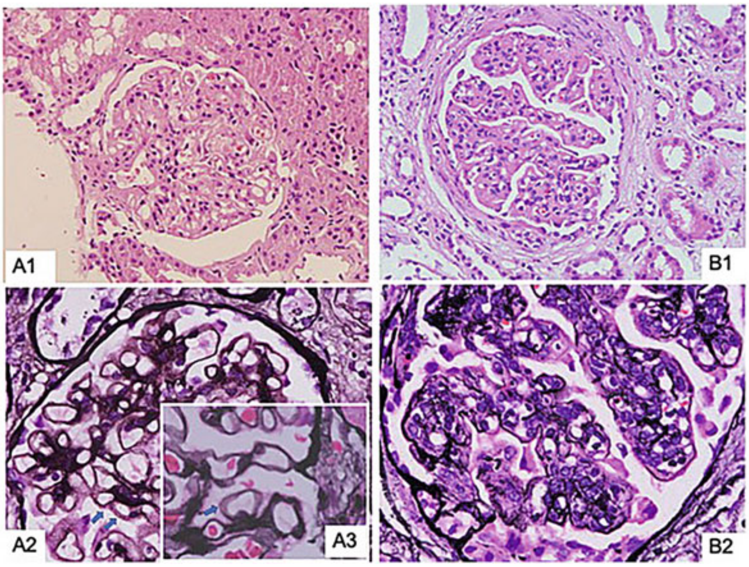

Fig. 4 Histological lesions typically associated with infection with hepatitis B (HBV) and hepatitis C (HCV) infection in children. (A) Membranous nephropathy in a 4-year-old boy with HBV DNA $>10^6$ copies per mL. H&E-stained glomerulus (A1) shows prominent glomerular basement membrane with stiff capillary walls. Silver methenamine stained glomerulus indicates "spikes" (arrows in A2) and numerous pinpoint "holes" (arrows in the inset A3) in tangential section, caused by the projections of basement membrane matrix towards the urinary space. (B) Membranoproliferative glomerulonephritis in a 12-year girl with HCV presenting with acute nephritic syndrome, nephrotic range proteinuria, and low complement C3. Hematoxylin and eosin-stained glomerulus in (B1) shows lobular accentuation, mesangial expansion, mesangial and endocapillary hypercellularity, while silver methenamine stained glomerulus in (B2) highlights the irregular capillary wall thickening with segmental glomerular basement membrane double contours. *Courtesy: Dr Geetika Singh, Department of Pathology, All India Institute of Medical Sciences, New Delhi, India.* A1 - B1 = x 200 magnifications; A2 - B2 = x400 magnifications

MN [54]. Contrary to primary MN that typically shows IgG4 deposition, lesions in HBV-associated MN contain IgG1 [55]. Presence of segmental lesions, mesangial proliferation, subendothelial or mesangial deposits, and/or tubular reticular inclusions suggest HBV-associated glomerulopathy [52, 56]. Circulating anti-phospholipase A2 receptor antibodies (anti-PLA2R), previously considered pathognomonic for primary MN, were found in a majority of patients with HBV-associated MN, and colocalized with HBV antigens within glomeruli [55].

Presentation

HBV is the major cause of MN in boys under 12 years of age. Children acquire HBV through vertical and horizontal transmission, and may not always have a history of hepatitis or active liver disease. Circulating immune complexes containing HBsAg and anti-HBs antibodies are frequently detected in the circulation, and serum levels of C3, C4, and total hemolytic complement may be decreased. While patients with MN present with nephrotic syndrome, those with MPGN have features of nephritis with or without heavy proteinuria [47]. Co-infection with HCV must be excluded in patients presenting with AKI, nephrotic syndrome and/or systemic vasculitis with hypocomplementemia and cryoglobulinemia [52]. Compared to primary PAN, HBV-associated PAN usually has worse vasculitis scores, milder skin lesions, mesenteric rather than renal involvement, and favorable response to therapy [52].

Management

As cytotoxic T cells and natural killer cells play a key role eliminating infectious hepatocytes, drugs that induce immune suppression may accelerate the progression of this disease [19]. Antiviral therapy leads to resolution of HBV-associated MN, MPGN, and/or type 3 cryoglobulinemia; monotherapy in patients that are immunosuppressed is not recommended and may be counterproductive [57]. KDIGO guidelines recommend that patients with HBV associated MN receive therapy with either interferon (IFN) or oral nucleotide (tenofovir, adefovir) or nucleoside (lamivudine, entecavir, telbivudine) reverse transcription inhibitors [57]. Conventional or pegylated IFN-α, approved for use in children ≥1-yr and ≥ 3-yr old, respectively, require subcutaneous administration for 24 weeks and 48 weeks, respectively [57]. While resistance to therapy is rare, adverse events are common and IFN-α is contraindicated in decompensated liver failure. Among oral agents, entecavir and tenofovir are preferred to lamivudine, since the latter carries high risk of resistance due to the YMDD mutation in HBV DNA polymerase [58]. Finally, the optimal duration of therapy with oral agents is uncertain, most agents are not licensed for use in children, and dose adjustments are necessary in patients with renal dysfunction [57].

Outcome

The prognosis of HBV-related MN is more favorable in children than adults, with rates of kidney failure estimated at <5% versus 25%–35%, respectively [52]. Histological lesions often resolve upon clearance of circulating HBeAg, whether spontaneously or after therapy.

Hepatitis C Virus (HCV)

Epidemiology

HCV virus is an enveloped positive sense single-stranded RNA virus of the family Flaviviridae. Infection with HCV affects 3% of the population globally, including 0.15% of children [59]. Children become infected through contaminated blood transfusions or vertical transmission and approximately 100,000 children in the United States are infected chronically with HCV [19]. Both the prevalence and genotype distribution vary markedly geographically [59]. While 75–90% of incident infections cause chronic viremia, this figure will likely decline with the use of direct-acting antivirals (DAA) [59]. Occult HCV infection is present in 30%–50% of cases with idiopathic and secondary glomerulopathies [60]. In some cases (5–10%), patients are co-infected with HIV-1, and their treatment is more challenging.

Pathology

The most common HCV-related GN is type I membranoproliferative GN (Fig. 4B), which may be accompanied by type 2 (80%) or 3 (20%) cryoglobulinemic vasculitis, typically in adulthood, after 10–15 years of acquiring HCV infection [47, 61]. MN is the second most common glomerulopathy. Chronic HCV infection might also cause tubulointerstitial injury [47] and/or acute tubular necrosis with hepatorenal syndrome [62], and increases risk of progression of CKD.

Pathogenesis

Similar to infection with HBV, glomerular injury requires active viremia. Cases of polyarteritis nodosa have been seen in people with subacute or chronic HCV viremia. Alternatively, other glomerulopathies (immune complex membranoproliferative GN, Ig A nephropathy, MN, FSGS, mesangioproliferative GN, fibrillary or immunotactoid glomerulopathies, or cryoglobulinemic TMA) are also associated with periods of chronic viremia. Usually, the glomerular lesions contain deposits, on or along the glomerular basement membrane, containing large lattice-like immune complexes, comprised of monoclonal IgM_K binding to the Fc portion of non-neutralizing, HCV-specific, IgG1 or IgG3; these IgGs ineffectively bind to certain HCV proteins (e.g., E2, NS3 or HCV RNA) [47]. Type 3 cryoglobulinemia, with circulating polyclonal IgM-polyclonal IgG immune complexes, may progress over 10–15 years to type 2 cryoglobulinemia, characterized by monoclonal production of IgM_K [48]. This overproduction of RF-IgM_K is caused by repeated B cell activation due to continued production of HCV-specific IgG to clear millions of HCV-IgG immune complexes daily, and/or molecular mimicry of host autoantigens by HCV core proteins NS5A and NS

[63]. Reactivity of IgM_K to IgG leads to rheumatoid factor activity.

Direct viral infection of glomerular and tubular cells can be demonstrated by immunochemistry, in situ hybridization and/or extraction of HCV RNA sequences [47, 48]. Disease pathogenesis may involve multiple pathways including increased expression of toll-like receptors by mesangial cells and/or generation of pro-inflammatory cytokines, insulin resistance with intrarenal production of insulin-like growth factor-1 and transforming growth factor-β, upregulated mesangial cell expression of angiotensin II type 1 receptors, and/or oxidative injury [47, 48].

Presentation

Patients with chronic HCV infection may have proteinuria, microscopic hematuria, hypertension or renal dysfunction on screening, or present with nephrotic syndrome, acute nephritis, and/or rapidly progressive GN [47, 59, 61]. Patients may have features of systemic vasculitis (purpuric rash, arthralgias) or glomerulonephritis in the presence of circulating cryoglobulins, which is termed mixed cryoglobulinemia syndrome [63]. Patients with decompensated liver disease may present with oliguric AKI.

Evaluation

KDIGO recommends that all HCV-infected patients should be screened annually for hematuria, proteinuria and reduced GFR, and undergo kidney biopsy if any of these findings persist [64]. Biopsy is also indicated in HCV-infected individuals presenting with acute nephritic syndrome, nephrotic syndrome and/or features of vasculitis. Conversely, all patients newly diagnosed with CKD should be screened for antibodies to HCV using an enzyme-linked immunoassay and qNAT for active viremia should be performed in patients with a positive immunoassay [64, 65]. Rarely, the anti-HCV immunoassay is negative while viremia is present, as may happen in early acute infection and/or in immunosuppressed hosts [64].

Management

The treatment of HCV-associated kidney disease focuses on eradicating HCV viremia, the source of nephritogenic immune complexes. Oral DAAs against HCV are now the therapy of choice since they enable sustained virological response in over 90% cases, across all genotypes, within 4–8 weeks [66, 67], and eliminate cryoglobulinemia [68]. Hence, the guidelines from KDIGO [64]; the European Association for the Study of the Liver (EASL 2018) [69]; the American Association for the Study of Liver Diseases/Infectious Diseases Society of America (AASLD/IDA) [70]; and the Sweden guidelines [71], all recommend therapy with DAAs for all HCV-associated glomerular diseases, particularly MPGN. Therapy with interferon-alpha (IFN-α) is no longer recommended as it is associated with low rate of virological response (<65%), non-sustained remission, need for prolonged therapy (48-weeks), poor patient tolerance and, in allograft recipients, risk of humoral rejection [66, 67].

Patients with HCV-associated glomerular disease with stable kidney function and non-nephrotic range proteinuria are initially treated with DAAs, usually in a combination of agents targeting different nonstructural proteins (NS5A, NS5B, and NS3/4A), for a duration of 12-weeks [66, 67]. Dose modification for decreased GFR is often required. Evidence on the safety and efficacy of DAAs in children is limited to combinations of sofosbuvir with ledipasvir, sofosbuvir with ribavirin and glecaprevir with pibrentasvir, all of which are approved for therapy in patients older than 12 years. Patients presenting with rapidly progressive kidney failure, acute cryoglobulinemia or nephrotic syndrome are candidates for additional measures to clear circulating immune complexes or alleviate inflammatory symptoms, such as immunosuppression and therapeutic apheresis [47, 48, 64]. Therapy with rituximab is more effective than conventional immunosuppression in prospective controlled studies in adults with cryoglobulinemic vasculitis or diffuse membranoproliferative GN, but carries risk of reactivation of HCV infection [64, 72].

Outcomes

Meta-analyses suggest that IFN-α therapy leads to reduced proteinuria and improved eGFR, although as mentioned, this therapy is no longer suggested [73]. Recent studies indicate sustained virological response in 95% of patients treated with DAAs, along with remission of kidney disease, reduced rate of decline of GFR and/or regression of glomerular lesions, particularly if therapy is initiated early [74].

Viral Hemorrhagic Fevers

Dengue Virus

Dengue virus is a positive sense single-stranded RNA virus of the *Flaviviridae* family that causes dengue fever, dengue hemorrhagic fever (DHF) and/or dengue shock syndrome (DSS). Infection is caused by one of four serotypes, is transmitted by the female *Aedes aegypti* mosquito, and is endemic in Southeast Asia, Latin America, and the Pacific Islands. Classic dengue usually resolves spontaneously and is not lethal; however, patients with severe infection are at risk of multiorgan dysfunction and AKI [75, 76]. AKI is observed in 0.9–10% of admitted children and 2–36% adults and is chiefly caused by ischemic hypoperfusion, acute inflammatory response with capillary leak, rhabdomyolysis and/or nephrotoxic drugs used to treat disease [75, 77]. Abnormalities in serum electrolytes (hyponatremia, hypokalemia) and the urine (proteinuria, microscopic hematuria) are observed in 20–80% patients, particularly in those with severe disease [75, 78]. Hyponatremia, which is tenfold more frequent than in non-dengue febrile illnesses, is typically from the syndrome of inappropriate anti-diuretic hormone release, intravenous overhydration and/or use of hypotonic fluids [78]. Glomerulonephritis with mesangial hypercellularity or diffuse proliferation may occur from direct viral cytopathic effects and/or immune or IgM deposition [77]. Rarely, thrombotic microangiopathy is reported [79].

Diagnosis of dengue infection is based on detection of NS1 antigen (<5 days) or IgM antibody (beyond day 4) by ELISA in the appropriate clinical context [75]. Laboratory abnormalities include increased hematocrit, severe thrombocytopenia, leukopenia, relative lymphocytosis, and/or abnormal coagulation parameters. No specific antiviral treatments are available and as such management focuses on fluid resuscitation and replacement titrated to maintain hemodynamic parameters and normalization of hematocrit, as per WHO guidelines [76]. The presence of AKI increases the risk of mortality in patients with DSS/DHF from 12–44% to >60% [75, 80]. Fluid overload is common in severe DSS and is the most common indication for kidney replacement therapy [75, 78]. Immunosuppression should be avoided in patients with glomerulonephritis; most patients recover with supportive care [75, 77].

Yellow Fever

Yellow fever virus is caused by a positive sense single-stranded enveloped RNA virus of the *Flaviviridae* family which is transmitted by the mosquito *Aedes aegypti* [81]. The infection, which endemic in certain tropical countries in Africa and South America, presents as an acute febrile illness that may progress rapidly to have hemorrhagic features and multiorgan dysfunction [82]. Patients with hemorrhagic manifestations, liver injury, disseminated intravascular coagulation, and shock are at risk of severe disease and oliguric AKI, which is manifested as acute tubular necrosis [82]. Diagnosis relies on isolating the virus by PCR and/or demonstrating serum IgM; liver biopsy, if performed, shows characteristic Councilman bodies. Management is chiefly supportive [81].

Ebola Virus

Like other Filovirus, Ebola is an enveloped, non-segmented, negative-sense RNA virus. This virus has been associated with outbreaks of fatal hemorrhagic disease in Africa since the 1970s, and an epidemic in 2013–16 leading to 11,000 fatalities in western Africa [83]. Disease course comprises of a prodromal phase, followed by a febrile period, then gastrointestinal symptoms, and finally, severe hypovolemic and septic shock, respiratory failure, and multiorgan dysfunction, with only minor bleeding manifestations [83, 84]. Patients with multiorgan dysfunction are at high risk of AKI and mortality [85]. Case fatality rates of 40–90% are reported at extremes of age and in resource-constrained settings [83, 84]. With no specific antiviral therapy, supportive care focuses on timely provision of cardiorespiratory support, treating/preventing hypovolemia, correction of electrolyte and metabolic abnormalities, and if indicated, kidney replacement therapy [83, 84]. The FDA recently approved a vaccine for Ebola, which is given as a single dose, and appears to be safe and protective against Zaire ebolavirus which has caused deadly Ebola outbreaks.

Hantavirus

Hantavirus is a negative sense enveloped RNA viruses belonging to the *Bunyaviridae* family and *Orthohantavirus* genus. These viruses are transmitted to humans by exposure to aerosolized rodent urine, feces, or saliva [86]. Human disease, reported with about 30 different hantaviruses across the world, has five phases, including febrile, hypotensive, oliguric, polyuric, and convalescent phases, with severity varying by virus type [86]. Transient nonselective proteinuria and microscopic hematuria are common findings in infection with all hantaviruses [86]. AKI is reported chiefly with hantavirus cardiopulmonary syndrome in United States and South America (Sin Nombre and Andes viruses) and with hemorrhagic fever and renal syndrome (Hantaan, Dobrava-Belgrade, Seoul, and Puumula viruses) in Asia and Europe [87]. AKI associated with Hantavirus is usually mild, except in infection with Hantaan or Dobrava viruses [86, 87]. When AKI occurs, GFR typically falls abruptly due to altered hemodynamics mediated by increased vascular permeability, akin to septic shock. Other contributors to renal injury include endothelial injury, complement activation, cytokine-mediated tubular, and interstitial damage [88]. Kidney biopsy typically shows either acute interstitial nephritis or acute tubular necrosis, with or without interstitial edema and hemorrhage [89].

Diagnosis is usually made by demonstrating IgM antibodies (within ≤ 3 weeks), a four-fold rise in IgG titers, or amplification of viral RNA in blood cells or serum by nested reverse-transcriptase PCR [90]. Management focuses on ensuring cardiorespiratory support; therapy with ribavirin may ameliorate disease if initiated early [91] but is ineffective when delayed to cardiopulmonary phase [92]. Kidney replacement therapy is occasionally required for patients with severe fluid overload or electrolyte abnormalities.

Congo-Crimean Hemorrhagic Fever (CCHF)

CCHF is caused by infection with a tick-born single-stranded RNA virus (*Nairovirus*) of the B*unyaviridae* family. This virus causes a frequently lethal viral hemorrhagic fever in Western Asia, the Balkans, Southern Europe, and most of Africa. Patients may become lethargic and develop hepatic and kidney failure. Ribavirin and immunoglobulins have been used in several cases but controlled studies are required to prove their efficacy in reducing mortality [19] .

Rift Valley Fever Virus (RVFV)

RVFV is a negative-sense single-stranded RNA virus from the family *Bunyaviridae* transmitted via mosquito vectors [93]. It is a zoonotic pathogen that primarily affects ruminants but can be lethal in humans. Although the initial cases were restricted to sub-Saharan Africa, most recent outbreaks were detected in the Middle East, raising concerns that the virus could spread to Europe and Asia. The infection can present as a mild self-limiting illness in many cases, or as a vasculitis with renal involvement or fatal hemorrhagic illness less frequently [19]. There are no specific treatments for RVFV.

Lassa hemorrhagic fever is produced by the Lassa virus, a segmented negative sense single-stranded RNA arenavirus, which is endemic in West African countries [94].The annual incidence of infection in West Africa is between 300,00 and 500,000 cases. Many of those infected by the virus do not develop symptoms, but approximately 10% can develop a fulminant hemorrhagic fever, with proteinuria, hematuria, and acute kidney injury, resulting in ~5000 deaths per year. Ribavirin given early in the course of the illness along with intensive fluid replacement and hemodynamic support appear to reduce the mortality of this disease [19].

Marburg Virus

The Marburg virus is a non-segmented negative sense RNA virus belonging to the order of Mononegavirales. The reservoir host of Marburg virus is the African fruit bat *Rousettus aegyptiacus.* The Marburg virus causes hemorrhagic fevers in humans and nonhuman primates [19]. The initial outbreaks were recognized in Marburg and Frankfurt Germany, Belgrade, and Serbia. This disease is associated with hypotension, extracellular fluid volume contraction, and coagulation disorders, leading to fulminant shock if not treated with aggressive hemodynamic support. There is currently no specific treatment or vaccine.

Argentine Hemorrhagic Fever (AHF)

AHF is a rodent-borne illness caused by the Junin virus that is endemic in the humid pampas of Argentina [95]. The Junin virus is an arenavirus that contains a bisegmented negative sense RNA genome, and spreads through aerosolization of host rodent excreta. Most infections result in clinical disease (80%), with a fatality rate of 15% to 30% if left untreated. During the first phase of the illness patients show leukopenia and thrombocytopenia, proteinuria, and hematuria. Liver enzymes are elevated, but BUN and serum creatinine are normal in the absence of dehydration or shock [95]. Specific therapy against AHF includes infusion of immune plasma during the prodromal phase of illness. However, there are no effective therapies for severely ill patients in the neurological-hemorrhagic phase. Ribavirin has been used in place of immune plasma in the early stages. A live attenuated vaccine is available that has reduced the incidence of AHF. Machupo virus, closely related to Junin virus, is found in Bolivia and can also causes prominent neurological symptoms and systemic hemorrhagic features, leading to shock and acute kidney injury in the worse cases [19].

HIV-Associated Nephropathies

Epidemiology: Approximately 2.8 million children under 19 years of age were living with HIV in 2019 [96]. Every day in 2019 approximately 880 children became infected with HIV-1 and 310 children died from AIDS-related causes, mainly because they did not have access to appropriate care [96]. Before the modern antiretroviral therapy (ART) era, persistent proteinuria and severe forms of microvascular thrombotic microangiopathies (TMAs) were frequently seen in adults and children with advanced HIV disease [97–99]. The incidence of these diseases fell dramatically to <10% and <0.5%, respectively, in subsequent years with the use of modern ART [99–101]. However, children need to receive continuous ART throughout their childhood to remain in good health, which creates significant public health challenges all over the world. Unfortunately, almost half of the children living with HIV worldwide are not receiving appropriate ART and as such, more than 100,000 children have died of an AIDS related illness since 2018 [96]. In addition, we continue to see children developing HIV-associated nephropathy (HIVAN) in the United States [101].

Over 80% of all children living with HIV reside in Africa. People from African ancestry have an increased risk of developing HIV-associated nephropathy (HIVAN) and other HIV-chronic kidney diseases (HIV-CKDs) [101, 102]. This increased risk has been attributed to Apoliprotein-1 (APOL1) genetic variants G1 and G2 [103]; these variants increase the lifetime risk of young adults who are not receiving appropriate ART to develop HIVAN by ~50% [104]. However, the relative prevalence of these APOL1 risk alleles is low, so most children living with HIV develop HIVAN independently of these risk variants [105–108]. Therefore, other unknown yet genetic factors associated with the African ancestry likely have additional roles precipitating HIVAN.

Pathology

HIV-associated kidney diseases have been recently classified into three main pathological categories [109]: (1) Glomerular-dominant disorders, including podocytopathies and immune complex-mediated glomerular diseases; (2) Vascular-dominant disorders, including TMA and an atypical form of HUS; and (3) Tubulointerstitial-dominant disorders due to ischemia/hypovolemia, nephrotoxic drugs, including ART (i.e., tenofovir, indinavir, aminoglycosides, amphotericin B, foscarnet, cidofovir, trimethoprim-sulfamethoxazole, and acyclovir), renal infections with bacteria, fungi, protozoans, and many of the viruses discussed above (HCV, BK, adenovirus, and/or herpes viruses).

Four subtypes of podocytopathies have been described [109]: (1) Classic HIVAN (defined as CG and tubular microcyst formation with interstitial inflammation and tubular injury); (2) Focal segmental glomerulosclerosis (FSGS) not otherwise specified (NOS) (defined as the presence of non-collapsing FSGS, with less prominent tubulointerstitial disease); (3) Diffuse mesangial hypercellularity; and (4) Minimal change disease (rare). All of these renal diseases show podocyte foot process effacement and proteinuria, without significant immune complex deposition. Pathologists recommend distinguishing classic HIVAN from FSGS (NOS), since HIV-causality can be proven in classic HIVAN [109]. However, ART can mask the typical presentation of classic HIVAN, and young children are less likely to develop CG and instead frequently develop mesangial hyperplasia and/or FSGS in combination with microcystic tubular lesions leading to renal enlargement (Fig. 5). Therefore, the diagnosis of childhood HIVAN should take into consideration the age of the child, as well as the typical clinical and renal histological features characteristic of this renal disease.

Several types of HIV-associated immune-complex kidney diseases (HIVICD) have been reported in adults and children living with HIV [110]. These diseases include MPGN, post-infectious GN, GN associated with mixed cryoglobulinemia, IgA nephropathy, lupus-like GN (with negative lupus serology and lack of lupus symptoms), MN, and renal diseases with a mixed histological pattern consistent with both HIVAN and HIVICD [109]. A large number of cases of HIVICD have been reported in South African children with proteinuria (~50%) [110]. Thus, given the heterogenous spectrum of all HIVICD renal diseases and the difficulties in establishing HIV causality, pathologists have recommended to replace the term “HIV immune complex kidney disease” with the specific description of the type of immune complex disease in the setting of HIV [109].

Several cases of TMA were reported in people living with HIV during the early years of the AIDS epidemic [98, 111]. In many cases, the TMA lesions were asymptomatic and were detected during autopsies [112]. Endothelial injury appears to be the

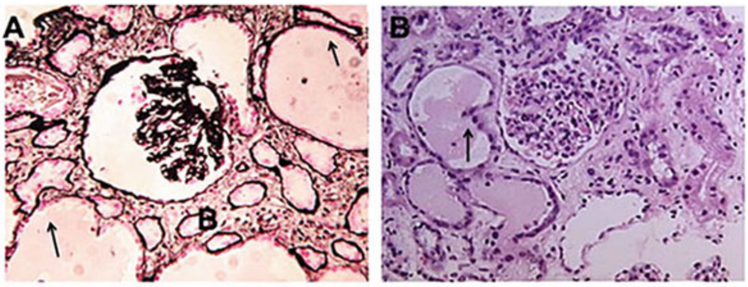

Fig. 5 Panels A and B show representative kidney sections from children with HIVAN. Panel A shows a kidney biopsy with collapsing glomerulopathy stained with periodic acid Schiff. Panel B shows a kidney biopsy with mesangial expansion and enlarged glomeruli stained with hematoxylin and eosin. Both panels show the tubular microcystic changes characteristics of childhood HIVAN (arrows) (both x200 magnifications)

primary event in the pathogenesis of TMA, causing the activation and deposition of platelets in arterioles and capillaries. In contrast, the atypical cases of HIV-HUS reported in young children during the pre-ART era [113], revealed TMA lesions characterized by the accumulation of fibrin in combination with glomerular and tubulointerstitial proliferative changes [113]. A deficiency in the von-Willebrand factor cleaving protease ADAMTS13 was found in one adolescent HIV+ female with thrombotic thrombocytopenic purpura (TTP) who developed a hemorrhagic stroke [114]. These TMA lesions were characterized by the intravascular accumulation of large vWF-fragments and platelets rather than fibrin.

Pathogenesis

HIV-1 is an enveloped double-stranded RNA virus. Retroviruses such as these carry the enzyme reverse transcriptase, which allows the virus to copy RNA into DNA to infect human cells. Most of our knowledge related to the pathogenesis of HIVAN is based on studies done in HIV-transgenic (Tg) mice or rats [102]. These models provided definitive evidence that the expression of HIV-transcripts in renal epithelial cells play a crucial role in the pathogenesis of HIVAN. However, both the infection and viral replication processes are bypassed in transgenic mice/rats and a key event that was not apparent from these rodent studies was how renal epithelial cells become infected in vivo. Renal epithelial cells do not express the CD4 molecule, which is the major HIV-1 receptor, and must use an alternative mechanism to become infected. One study done in 1998 showed that primary HIV isolates derived from children with HIVAN were able to induce a CD4 independent low level productive infection of renal tubular epithelial cells cultured from the urine of children with HIV-CKDs [98]. This study demonstrated that infected renal epithelial cells were able to transfer viruses to co-cultured HIV-negative mononuclear cells even after 20 days in culture [98]. In addition, this study showed that the most effective way to infect cultured renal epithelial cells is through a “Trojan horse” like mechanism. In this process, HIV+ mononuclear cells established close contact with renal epithelial cells, and released viruses into a “virological synapse” space formed between the cells. Subsequently, HIV is taken up by the epithelial cells via endocytosis (Fig. 6), and through mechanisms that are not clearly understood, induces a low-level and transient productive infection in these cells [98]**.** Viral transcripts in podocytes and tubular epithelial cells have been detected in adults with HIVAN [115], and these studies hypothesize that the kidney might become an HIV-reservoir independent from the blood compartment [116]. Other studies demonstrated that transmembrane-TNF-α (tm-TNF- α) plays a key role facilitating the infection of CD4 negative podocytes and tubular epithelial cells by increasing the uptake of viral particles via clathrin-mediated endocytosis, and by increasing the production of viral transcripts via the activation of NF-kB [117]. Heparan sulfate proteoglycans (HSPG) also facilitate the infection of renal epithelial cells by increasing the attachment and endocytosis of HIV-1 [117]. Interestingly, tm-TNF-α can act both as a ligand, releasing soluble TNF-α that activates the TNF receptors, and as a receptor, facilitating the formation of virological synapses between mononuclear and kidney epithelial cells [102]. These findings, together with the high TNF-α plasma levels found in young HIV-Tg_{26} mice and children with HIVAN, support the notion that TNF-α plays an important role in the pathogenesis of childhood HIVAN [102].

Another unique feature of HIVAN is the finding of de-differentiation and proliferation of podocytes and tubular epithelial cells leading to the collapse of glomerular capillaries and formation of tubular microcysts and renal enlargement. Previous studies done in cultured murine epithelial cells immortalized with the SV-40 large T antigen showed that HIV-Nef increased the proliferation of these cells [118], supporting that HIV-1 can induce direct proliferative changes in renal epithelial cells. However, these findings were not reproduced in cultured primary human renal epithelial cells. Furthermore, cultured human renal epithelial cells infected with HIV-1 showed poor viability and proliferative capacity [98]**.** In contrast, cytokines and heparin binding growth factors induce significant proliferation of primary renal epithelial cells cultured from

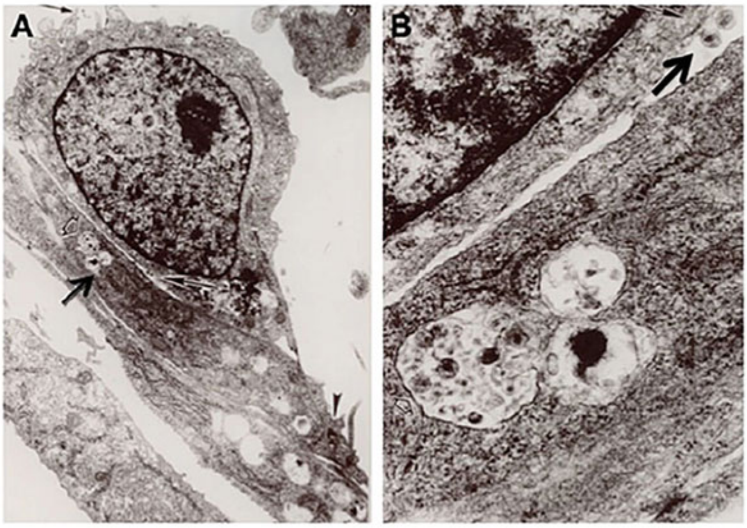

Fig. 6 Electron micrographs showing a mononuclear cell on top of renal tubular epithelial cells (RTECs) cultured from a child with HIVAN. The arrowhead in panel A points to a tight junction between RTECs. The arrow points to HIV viral particles inside the RTEC. Panel B shows a higher magnification of the viral particles inside the RTEC, as well as viral particles in the space between the mononuclear cell and RTEC (arrow). Original magnification x10,000 (A) and x50,000 (B)

HIV-Tg$_{26}$ mice and children with HIV-CKDs [102, 119]. In fact, both HIV-Tg$_{26}$ mice and children with HIVAN had up-regulated expression of renal heparan sulfate proteoglycans (HSPG) that facilitate the recruitment of the HIV-Tat protein [102] and other heparin binding growth factors. Considering that podocytes are terminally differentiated cells, it has been postulated that they may detach or die when forced to enter the mitotic cell cycle under the influence of growth factors [120]. Injured podocytes appear to be replaced by less differentiated parietal epithelial or progenitor cells, that appear to be highly sensitive to the mitogenic effects of heparin binding growth factors [102]. In support of this notion HIV-Tat, acting in synergy with FGF-2 and other heparin binding growth factors, can disrupt the cytoskeletal structure of podocytes and glomerular endothelial cells, and precipitate HIVAN in young HIV-Tg$_{26}$ mice [121–123]. Finally, other cytokines that are released by HIV-infected cells, including TNF-α and IFN-γ, can induce the expression of APOL1 in renal epithelial cells [124]. Since up-regulated expression of the APOL1 risk variants G1/G2 reduces the viability of renal epithelial cells [124, 125], many speculate that HIV may act as a second hit to precipitate HIVAN by increasing the cytotoxicity of APOL1 in podocytes, rather than by inducing direct proliferative changes in these cells.

The pathogenesis of HIVICD kidney diseases in the setting of HIV is not well understood, and varies depending on each of the clinical forms described above. In general, HIV infection is associated with activation and dysregulation of the immune system that facilitates the formation of immune complexes and their deposition in the kidney. Co-infections with other agents may exacerbate HIVICD. Renal deposits of IgM, IgG, IgA, and complement (including C3, C4 and C1q) are typically found in these cases [126]. Interestingly, renal biopsies done in South Africa showed “a ball in cup” appearance of subepithelial immune deposits in the glomerular basement membrane by electron microscopy, although these lesions have not been reported in other studies [110]. Tubulo-reticular inclusion bodies are detected in HIVICD as well as HIVAN, hepatitis C and lupus-like nephritis [126]. One case report involving two white men suggested that HIV-1 can have a direct role in the pathogenesis of IgA nephropathy [127]. Circulating anti-HIV IgG and IgA antibodies were detected in the circulation of both patients, and isolated from the renal biopsy of one of them [127]. HIV p24 and gp120 antigens were also isolated from the renal tissue of both patients [127]. However, it remains unclear whether IgA nephropathy in the setting of HIV is due to the deposition of IgA directed against viral antigens or from other causes. Finally, other secondary causes of HIVICD should be ruled out in all cases of MN (i.e., HBV co-infection, or anti-PLA2R autoantibodies) and membranoproliferative glomerulonephritis (i.e., HCV co-infection).

The pathogenesis of HIV-associated vascular diseases is multifactorial and characterized by the development of TMA lesions. Endothelial injury is the primary event leading to development of TMA and HUS in children living with a high HIV viral load. Chronic endothelial injury, activation, and increased leukocyte adherence have been detected in the aortic endothelium of HIV-infected patients [128]. Endotoxin, viral co-infections (e.g., CMV), immune complexes, and drugs are among many factors that can induce endothelial injury and activation in HIV+ people. A direct role for HIV-1 inducing endothelial injury and dysregulation is supported by the presence of the HIV-1 p24 antigen in endothelial cells [111]. HIV-productive infection of the vascular endothelium remains a controversial issue, since endothelial cells do not express CD4. Previous studies showed that HIV-1 can FUSE to cultured renal glomerular endothelial cells through a CD4 independent CXCR-4 mediated mechanism [129], but HIV transcripts have not been detected in glomerular endothelial cells. Alternatively, endothelial cells can be activated by HIV-cytokines and viral proteins, such as Tat and gp120 [122, 130]. In fact, the dramatic reduction in the incidence of TMA lesions, and markers of endothelial injury/activation during the modern-ART era [131], strongly suggest that HIV-1 plays a direct role in the pathogenesis of these lesions.

Presentation and Outcomes

Childhood HIVAN is a late manifestation of HIV-infection. Children with HIVAN usually have a clinical history of sustained high viral load and low CD4 counts for months. Isolated persistent proteinuria is the first clinical sign of HIVAN. Edema, hypertension, or azotemia, are rare in the early stages of HIVAN. All types of renal epithelial cells, urothelial cells, inflammatory cells, and/or casts can be detected in the urine, including urinary epithelial cells grouped together forming microcysts in fresh urine specimens before centrifugation [132]. Renal sonograms may show enlarged echogenic kidneys. Microscopic hematuria can be present, but is more frequently seen in children with HIVICD. In fact, children with macroscopic hematuria or microscopic hematuria without proteinuria, increased BUN or serum creatinine without proteinuria, and hematuria or proteinuria in White, Asian or Hispanic children, require a more extensive evaluation for the presence of other kidney diseases. A definitive diagnosis of HIVAN can only be made on a renal biopsy. Studies done in South Africa [110], showed that almost 50% of children with suspected HIVAN and persistent proteinuria actually had renal histological features consistent with other renal diseases. Limited long-term data are available to predict the outcome of HIV-CKDs during the modern ART era. A recent study showed that HIVAN had a worse prognosis than HIVICD [101]. It appears that once the clinical and renal histological features of HIVAN are well established, current treatments might not prevent progression to kidney failure [101]. However, even HIVICD cases have been progressing at a slower rate for several years [101]. In addition, many HIV+ children and adolescents of African descent treated with ART for years can still have mild but persistent reductions in GFR (< 80 ml/min/1.73 m^2), and are at high risk of developing chronic kidney failure in adulthood [101].

Atypical cases of HIV-HUS were reported in children in the pre-ART era. The presentation of HIV-HUS was insidious and followed a progressive clinical course to kidney failure or death due to infections and bleeding complications [113]. In another case, an adolescent female with HIV-TTP developed a stroke in the hospital, but was treated rapidly with ART and plasma exchange; she continued to improve with sustained virologic suppression and eventually had recovery of the platelet count and no major neurological or kidney sequela [114]. Thus, it appears that the early recognition and treatment of these cases can improve their outcome.

Management: Modern ART has substantially improved the clinical outcome of all children living with HIV kidney diseases [100, 101]. Nephrologists should work in close collaboration with HIV-experts in the treatment of these patients. Updated guidelines for the management of HIV-chronic kidney diseases [133], and the panel on antiretroviral (ARV) therapy and medical management of children living with HIV provide useful information for this purpose. ARV-naïve children and adolescents should begin their treatment with two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), plus one additional drug that could be a non-nucleoside reverse transcriptase inhibitor (NNRTIs), an integrase strand transfer inhibitor (INSTI), or a pharmacokinetic (PK)-enhanced protease inhibitor (PI). All NRTI, except for abacavir, are excreted by the kidney and their dosage should be adjusted based on kidney function. Integrase inhibitors and CCR5 antagonists can be used at similar dosages in children with impaired kidney function. Tenofovir disoproxil fumarate (TDF) is an effective ARV drug that shares the mechanism of action of NRTIs and is used widely to prevent and treat HIV/AIDS in combination with emtricitabine. However, it can induce renal tubular injury, isolated hypophosphatemia or glycosuria, Fanconi Syndrome, and/or bone disorders. For these reasons, it is not approved by the FDA in children under 2 years of age. Most recently, the FDA has approved the use of tenofovir alafenamide (TAF), which causes less renal toxicity and can be used in patients with GFR >30 ml/min/1.73 m^2. TAF appears to be as effective as TDF in achieving or maintaining virological suppression. In particular, children receiving NRTIS in combination with other nephrotoxic drugs (listed above when discussing the tubulointerstitial-dominant disorders in the setting of HIV), should be monitored more closely. Any change in serum creatinine levels, proteinuria, crystalluria, extracellular fluid volume, or acid-base status should alert physicians to the potential toxicity of these drugs. Overall, it remains to be determined how modern ART affects the renal outcome of children HIVICD, but maintaining a suppressed viral load appears to improve the outcome of these patients [101]. Angiotensin converting enzyme inhibitors (ACEi) are recommended for the treatment of proteinuria and hypertension in all children with HIV-CKDs [133]. Young children taking ACEi should be monitored more closely as they are at higher risk of becoming dehydrated secondary to diarrhea or vomiting, and developing AKI. In addition, they may develop salt wasting tubular disorders that put them a higher risk of becoming dehydrated when infected with other viruses. As ACEi can cause fetal malformations, they should be used with great caution in adolescent females with the potential for pregnancy. Steroids are not effective in children with HIVAN, but may provide benefits in children with undetectable viral loads and HIVICD [101]. Data regarding the optimal treatment with HIVICD are limited. The use of immunosuppressive drugs in children and adolescents living with HIV may exacerbate other infections, including tuberculosis in developing countries, and as such these risks should be weighed against potential benefits. Unfortunately, no randomized case-controlled studies have been performed in children with HIVICD and as such no definitive recommendations can be given regarding the use of steroids in these patients.

HIV+ children with end stage kidney failure should be treated with dialysis or kidney transplantation. The outcome of dialysis during the modern ART era has improved, but there are no studies comparing the outcome of HIV+ vs. HIV-children on dialysis. The presence of cardiovascular disease is associated with a higher mortality in HIV+ children undergoing dialysis [134]. Peritoneal dialysis is used more often in resource-limited countries, while hemodialysis is the preferred modality in the USA. The peritoneal fluid contains infections HIV-1 and should be handled with caution. Overall, the main priority should be to suppress the viral load in order to prevent infections and cardiovascular complications, and make these patients eligible for a kidney transplant. Successful kidney transplantation has happened in selected adult patients with undetectable viral loads, both in the United States and Europe. They have shown equivalent patient and allograft survival rates when compared to HIV- recipients [135, 136]. In the USA, no evidence of recurrent de novo FSGS, HIVAN, or nephrotic range proteinuria has been reported in HIV+ kidney recipients [135]. However, these patients do have higher rates of acute rejection, delayed graft function, and drug interactions. Finally, due to the limited availability of kidney donors, surgeons in South Africa and the USA are performing kidney transplants between HIV+ donors and HIV+ recipients with promising preliminary results [135, 137].

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Sickle Cell Nephropathy in Children

28

Thomas Graf, Connie Piccone, and Katherine MacRae Dell

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Abstract

Sickle cell disease (SCD) is one of the most common genetic disorders in the United States, affecting over 100,000 Americans and millions of people worldwide, primarily of African or Mediterranean descent. SCD is a group of hemoglobinopathies inherited in an autosomal recessive fashion. Nearly all organ systems are affected by SCD, including the kidneys. The renal manifestations of SCD are known as sickle cell nephropathy (SCN). Multiple pathogenic processes contribute to development of SCN, notably renal ischemia and hemolysis, which result in both glomerular and tubulointerstitial

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disease. Clinically significant complications of SCD include hyperfiltration, albuminuria, hyposthenuria, hematuria, hypertension, acute kidney injury (AKI), and chronic kidney disease (CKD). Despite these complications, occurrence of end-stage kidney disease (ESKD) is relatively uncommon, particularly in children. Of those who progress to ESKD, both dialysis and transplantation are viable treatments. While transplantation in SCN is associated with increased complications compared to other causes of ESKD, it is preferred over dialysis due to more favorable outcomes.

Keywords

Chronic kidney disease · Sickle cell disease · Sickle cell trait · Sickle cell anemia · Glomerular hyperfiltration · Albuminuria · Hypertension

Introduction

Sickle cell disease (SCD) is one of the most common genetic disorders in the United States, affecting over 100,000 Americans as well as millions of people worldwide, primarily of African or Mediterranean descent [1]. SCD is inherited in an autosomal recessive fashion. Sickle hemoglobin (HgbS) and other hemoglobin variants arose out of genetic selection due to the fact that heterozygosity for HgbS confers some resistance to malarial infection with a resultant survival advantage in endemic areas. All forms of SCD are the result of mutations in the two genes that encode β-globin, a major component of adult hemoglobin. The most common form of SCD is homozygous SS disease, also called sickle cell anemia (SCA). Other variants of SCD are the result of compound heterozygosity for HgbS and other β-globin variants, including SC, Sβ^+thalassemia, and Sβ^0thalassemia. All individuals who are homozygous or compound heterozygous for HgbS exhibit clinical manifestations of SCD. However, there is significant variability in clinical severity, primarily related to genotype. Patients with SS disease generally have the most severe phenotype followed by individuals with Sβ^0 thalassemia, whereas those with SC and Sβ^+ thalassemia tend to have milder clinical phenotypes.

HgbS polymerizes in the deoxygenated state and leads to red blood cell sickling, which, in turn, leads to the two major pathogenic processes of SCD, *vaso-occlusion* and *hemolysis*. Vaso-occlusion is associated with tissue ischemia, and hemolysis results not only in anemia but also in endothelial activation, inflammation, and vasculopathy. Nearly all organ systems are affected by SCD, leading to the well-recognized complications of pain crises, acute chest syndrome, infection (due to auto-infarction of the spleen early in life), and cerebrovascular accidents. The kidneys are susceptible to injury by multiple mechanisms that contribute further to the significant morbidity and mortality associated with SCD. The renal manifestations of SCD, which have been collectively termed “sickle cell nephropathy” (SCN), are summarized in Fig. 1. This chapter will address the current understanding of the pathogenesis, clinical presentation, management, and prognosis of SCN.

Pathogenesis and Pathology of Sickle Cell Nephropathy

The two major underlying pathophysiologic processes in SCD, vaso-occlusion and hemolysis, can result in both glomerular and tubulointerstitial involvement and damage, as illustrated in Fig. 2. In addition, medications utilized for the treatment of SCD, notably nonsteroidal anti-inflammatory drugs (NSAIDs) and chelating agents, can also cause kidney damage. In the last few decades, the mechanisms by which renal complications of SCD arise has been greatly facilitated by the availability of transgenic mice expressing sickle hemoglobin as well as intervention trials in both children and adults.

Glomerular Disease

Glomerular Disease Pathogenesis

The earliest manifestation of SCN is glomerular hyperfiltration, which is clinically evident as an

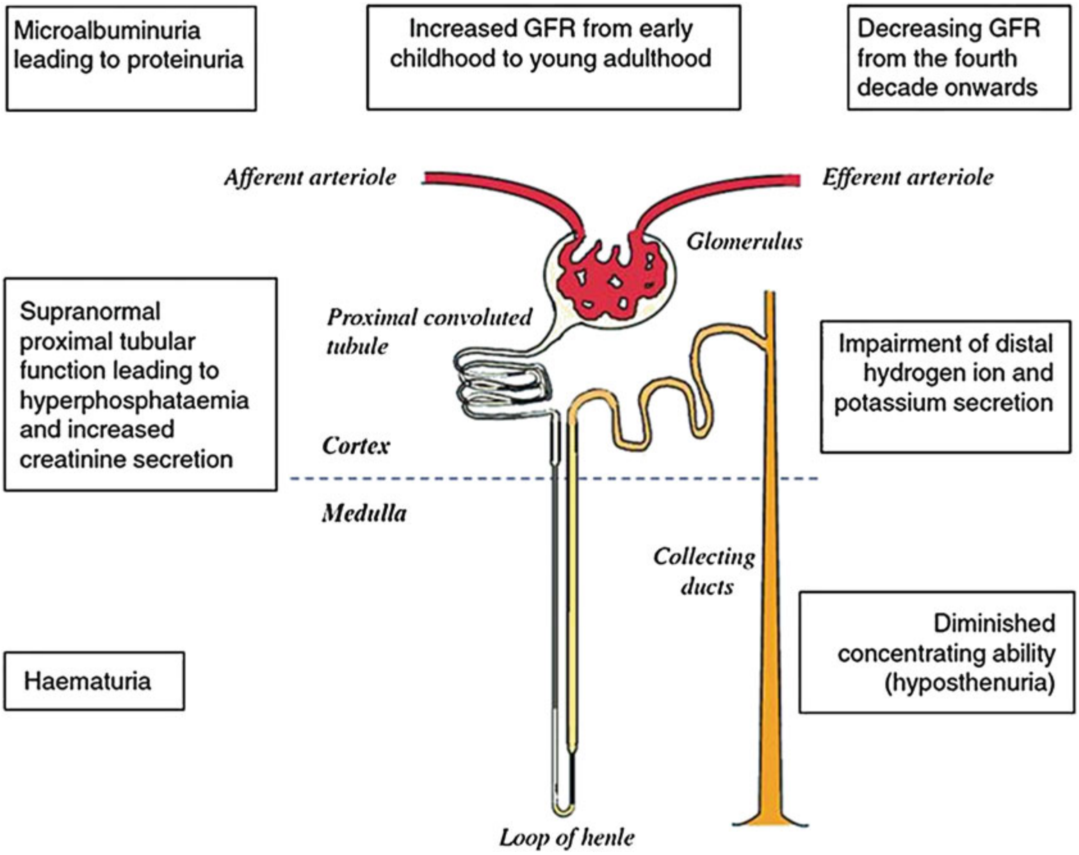
Microalbuminuria leading to proteinuria
Increased GFR from early childhood to young adulthood
Decreasing GFR from the fourth decade onwards
Afferent arteriole
Efferent arteriole
Glomerulus
Supranormal proximal tubular function leading to hyperphosphataemia and increased creatinine secretion
Proximal convoluted tubule
Impairment of distal hydrogen ion and potassium secretion
Cortex
Medulla
Collecting ducts
Diminished concentrating ability (hyposthenuria)
Haematuria
Loop of henle

Fig. 1 Renal abnormalities in sickle cell disease. A representation of the renal nephron demonstrating the regions damaged in sickle cell disease leading to the clinical manifestations of sickle cell nephropathy. *GFR* glomerular filtration rate. (Reprinted from Sharpe and Thein [51], with permission)

elevated glomerular filtration rate (GFR) starting in infancy in the vast majority of affected patients [2]. Multiple mechanisms for hyperfiltration have been proposed [3]. Animal models and patients with SCD demonstrate potentially reversible increase in renal blood flow and a decrease in renal vascular resistance. Localized prostaglandin (PG) release and increased nitric oxide synthase in response to hypoxia can also result in increased renal blood flow. The role of increased PG release as a key mechanism for the increased renal blood flow (RBF) and glomerular hyperfiltration seen in SCD is supported by the finding that indomethacin (a PG inhibitor) causes significant reductions in GFR and RBF in SCD patients but minimal or no decrease in healthy controls.

Hemolysis can also contribute to elevated GFR, via induction of heme oxygenase-1 (HO-1) in renal tubules, interstitial cells, and the vasculature as well as circulating endothelial cells [4]. HO-1 is the rate-limiting enzyme in the degradation of heme, converting heme to biliverdin, with resultant iron release and carbon monoxide (CO) production. Both biliverdin and CO are potent antioxidants, and CO acts locally as a vasorelaxant, thus increasing both the total RBF and GFR. Endothelin-1 (ET-1) has also been implicated in the progression of SCN due to its vasoconstrictive and pro-inflammatory effects. ET-1 is released in response to hypoxia, angiotensin II, and inflammatory cytokinesis, and it is increased in patients with SCD [5]. ET-1 leads to production of reactive oxygen species through the endothelin type A (ETA) receptor, the expression of which is upregulated in SCD mouse models. Animal studies suggest that treatment with an

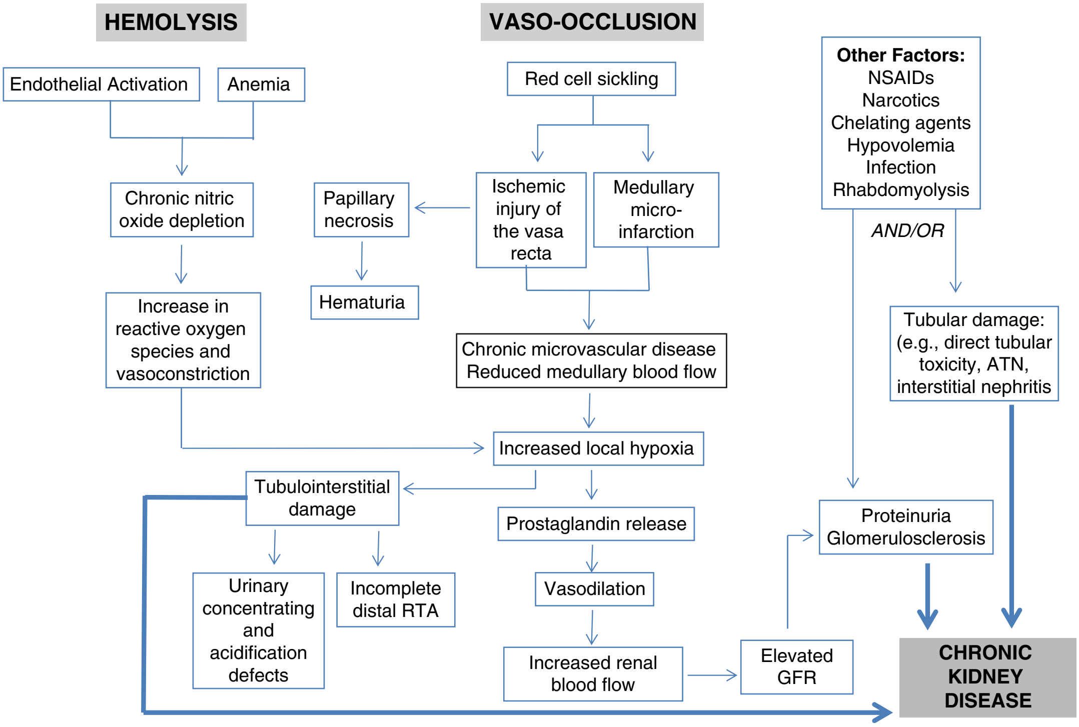
HEMOLYSIS
VASO-OCCLUSION
Endothelial Activation
Anemia
Chronic nitric oxide depletion
Increase in reactive oxygen species and vasoconstriction
Red cell sickling
Papillary necrosis
Hematuria
Ischemic injury of the vasa recta
Medullary micro-infarction
Chronic microvascular disease Reduced medullary blood flow
Other Factors: NSAIDs Narcotics Chelating agents Hypovolemia Infection Rhabdomyolysis
AND/OR
Tubular damage: (e.g., direct tubular toxicity, ATN, interstitial nephritis
Increased local hypoxia
Tubulointerstitial damage
Urinary concentrating and acidification defects
Incomplete distal RTA
Prostaglandin release
Vasodilation
Increased renal blood flow
Elevated GFR
Proteinuria Glomerulosclerosis
CHRONIC KIDNEY DISEASE

Fig. 2 Pathophysiology of sickle cell nephropathy. Flow diagram depicts the pathways by which renal abnormalities develop as sequelae of the two primary pathophysiologic processes in SCD (vaso-occlusion and hemolysis) or other factors (such as medications), each of which may lead to diminished renal function and chronic kidney disease. *GFR* glomerular filtration rate, *RTA* renal tubular acidosis, *NSAIDs* nonsteroidal anti-inflammatory drugs

ETA antagonist improves kidney function and reduces injury, especially in combination with hydroxyurea (HU) therapy. Whether ETA blockers, which are now being studied in clinical trials for a variety of proteinuric kidney diseases, will have similar effects on SCD patients has yet to be determined.

Glomerular Pathology in SCD

A spectrum of glomerular pathologies has been reported in a renal biopsy series of SCD patients with proteinuria [3, 6]. Four main glomerular pathologies have been associated with SCN: (1) glomerular hypertrophy without overt sclerosis; (2) focal segmental glomerulosclerosis (FSGS) and its variants (such as collapsing FSGS); (3) membranoproliferative glomerulonephritis (MPGN); and (4) thrombotic microangiopathy (TMA). Although FSGS was the most common finding in one adult series [6], in a biopsy cohort of 36 children aged 4–19 years with SCD, mesangial hypertrophy was the most common finding (75% of patients) followed by FSGS (30%), MPGN (16%), and TMA (2%) [7]. In the past, the term “sickle cell glomerulopathy” was used to describe the MPGN-like pathology, but more recently the term has been applied to FSGS. Given the variable use of this term, it is more appropriate to describe the specific pathology rather than using the nonspecific term “glomerulopathy.”

Tubulointerstitial Disease

Both renal ischemia and hemolysis contribute to the pathogenesis of SCN [3, 4]. Several factors make the renal medulla susceptible to injury in SCD, ultimately resulting in distal nephron dysfunction and damage. The relative hypoxic and acidotic environment of the renal medulla makes it particularly prone to sickling of red blood cells that traverse the medullary vasculature. The resultant focal vascular occlusion causes chronically reduced blood flow. Red cell sickling also leads to vaso-occlusion within the vasa recta, resulting in vessel damage. Chronic and acute vasocontriction results in endothelial injury, which causes release of pro-inflammatory cytokines. Animal and human data suggest that ischemia-reperfusion injury also contributes to disease pathogenesis in multiple organs, including the kidney [8]. Hemolysis also induces tubulointerstitial injury by release of free plasma hemoglobin, which is readily filtered and taken up by renal tubule cells, resulting in iron accumulation [4]. As with ischemia, hemolysis also induces a marked inflammatory response, resulting in upregulation of inflammatory and profibrotic cytokines.

Defective Urinary Concentrating Mechanisms

The most common renal complication in SCD patients is impaired urinary concentrating ability (hyposthenuria). Urinary diluting mechanisms are generally intact. Progressive loss of deep juxtamedullary nephrons with age was once thought to be the primary mechanism for hyposthenuria. However, more recent data from the BABY HUG study, a multicenter randomized control trial of hydroxyurea (HU) in young children, show that hyposthenuria occurs at an early age and is somewhat correctable with administration of HU [2, 9]. The mechanism for this early hyposthenuria remains undefined. However, adults with SCD do not show similar improvement in urinary concentration with initiation of HU, suggesting that nephron loss and tubulointerstitial damage eventually contribute to the pathogenesis.

Alterations in Potassium and Acid-Base Homeostasis

SCD patients may have altered potassium handling due to distal nephron dysfunction, but those with intact kidney function typically do not develop hyperkalemia [3]. Hyperkalemia may become evident, however, when patients are exposed to factors that may impact potassium secretion in the kidney, such as volume depletion or medications, including angiotensin-converting enzyme inhibitors (ACEIs). Impaired acid-base hemostasis has also been reported in SCD and can occur by several mechanisms. Urinary acidification requires intact distal nephron function, which can be compromised in situations of medullary ischemia in SCD. Ammoniagenesis (renal cell metabolism of glutamine to $NH4^+$ and HCO_3^-) may also be impaired in patients with SCD, especially those treated with NSAIDs. Although impaired responsiveness to both acid and potassium loads suggests a distal renal tubular acidosis (RTA), patients with SCD without CKD have typically been reported to have normal serum bicarbonate levels [3]. Two larger, more recent studies, however, demonstrated that mild metabolic acidosis may be more common than previously thought [10, 11]. Correlation of metabolic acidosis and markers of hemolysis suggest that even a relatively mild acidosis occurring as the result of tubular dysfunction in SCD patients could contribute to worsening hemolysis by increasing sickling and promoting red cell destruction.

Papillary Necrosis

Hematuria appears to result from red cell sickling in the medulla with subsequent vascular obstruction and red cell extravasation. Pathologically, papillary necrosis (PN) manifests as intact collecting ducts amid diffuse areas of fibrosis [12]. There is also evidence of repeated, small focal infarctions of the papilla, which ultimately affect local circulation.

Clinical Features and Treatment of SCN

Although the numerous renal manifestations of SCN detailed in this chapter have been well documented in the literature (hyposthenuria, hematuria, impaired urinary potassium excretion and acidification, glomerular hyperfiltration and tubular dysfunction, medullary carcinoma, and CKD), most, unfortunately, are without specific effective therapies or clear prognoses. There are, however, several areas in which expert recommendations about kidney disease assessment and management have been published [13]. In addition, several therapeutic trials are underway or have been completed that could significantly impact the progression of kidney disease (available at clinicaltrials.gov).

Glomerular Disease

Hyperfiltration

As reported by the BABY HUG study, the majority of children with SCD manifest an elevated GFR, consistent with hyperfiltration, as early as 9 months of age [2]. Hyperfiltration often persists through childhood into adulthood. In the HUSTLE study, a prospective study of the long-term effects of HU in 23 children with a median age of 7.5 years, over 80% of the children had a measured GFR >1 SD above the normal for age [14]. A series of over 400 adults with SCD and a median age of 30 years reported that 49% of subjects had hyperfiltration using a definition of estimated GFR (eGFR) >130 mL/min/1.73 m^2 for women and >140 mL/min/1.73 m^2 for men [15]. Treatment with hydroxyurea (HU) did not affect hyperfiltration in infants in the BABY HUG trial [9] but did lessen hyperfiltration (GFR measured by (99m)Tc-DTPA decreased from 167 ± 46 to 145 ± 27 mL/min/1.73 m^2 ($P = 0.016$)) in older children after 3 years in the HUSTLE study [14]. This decrease in GFR correlated with an increase in fetal hemoglobin and a decrease in lactate dehydrogenase.

Albuminuria

Hyperfiltration has been identified as a risk factor for developing albuminuria and CKD in multiple kidney diseases, including SCN [16, 17]. Although the majority of children with SCD have an elevated GFR consistent with hyperfiltration, only a subset will develop significant albuminuria. Microalbuminuria (MA), which is typically defined as urinary albumin/creatinine ratio of 30–300 mg/g creatinine, is a potential early sign of glomerular damage and may predict progression to clinically significant CKD. Up to 20% of children with SCD demonstrate MA on routine urine screening [18, 19]. Twenty-six percent of a cohort of 303 pediatric and adult patients with SS disease (mean age 21 years) exhibited MA [20]. The prevalence of MA in studies of adults with SCD is even higher, ranging from 30% to 60% depending on the population studied [20, 21]. In addition to hyperfiltration, severity of anemia in SCD and the presence of hemolysis have also been identified as important risk factors for albuminuria [11, 22, 23] in both adult and pediatric SCD, although some studies in adults have not found such associations [21, 24].

Expert consensus guidelines recommend yearly albuminuria screening of patients ages 10 years and older, although the evidence to support this recommendation is not strong [13]. The issue of screening for MA is complicated by the fact that MA in SCD can regress and remain unchanged in some patients. In a retrospective study of 373 children with SCD, 45 (12%) had MA at baseline, but over the 5-year course of the study, 24 patients developed new MA and 24 patients showed normalization of MA [19]. In a study of 175 pediatric and young adult SCD patients with serial MA measurements, 26% had baseline albuminuria, of whom approximately half developed persistent albuminuria [20]. Taken as a whole, these studies demonstrate that MA precedes development of persistent albuminuria in a subset of SCD patients but may also regress, making it a relatively insensitive biomarker for kidney disease progression in children.

Macroalbuminuria (>300 mg/g creatinine), also termed overt proteinuria, is less common, especially in children. In one study of more than 400 children with SCD, overt proteinuria (defined as urine dipstick ≥1+ for protein) was documented in 6.2% of children and 12% of adolescents [22]. Macroalbuminuria is reported to occur in about 26% of adults with HgbSS disease and 10% of those with other sickle cell related disorders [21]. Once macroalbuminuria develops, it invariably persists [20].

Several studies have shown a reduction in albuminuria in pediatric and adult patients taking HU [25, 26]. The use of ACEI in adult patients with albuminuria with or without hypertension is recommended by expert consensus [13]. Despite these benefits, it is less clear if ACEI use produces similar long-term benefits in children with albuminuria, especially those with MA.

Hypertension

Despite the presence of multiple mechanisms by which the kidney can be affected by SCD, hypertension has been thought to be relatively uncommon, and SCD patients often have been reported to have low to normal blood pressures [27, 28]. Emerging data, however, suggest that hypertension in SCD is more common than previously appreciated, including in children. Elevated in-office blood pressures occur in 8–10% in children with SCD [29, 30]. A study of 38 asymptomatic children with SCD who underwent ambulatory blood pressure monitoring (ABPM) found that approximately 40% met criteria for ambulatory hypertension [30]. Importantly, approximately 30% of patients studied demonstrated masked hypertension, which, by definition, would be missed on routine blood pressure readings during office or study visits. While the reported differences in rates of hypertension may reflect variations in the nutritional status, body mass index, and SCD severity of the patient population, the methodology for assessing blood pressure may also play a significant role. The high prevalence of masked hypertension suggests that clinicians should have a low threshold for performing ABPM on patients with SCD, especially those with evidence of renal involvement, such as albuminuria.

Children with SCD and elevated blood pressure may be at higher risk for development of target organ damage. In the Silent Cerebral Infarct Multicenter Clinical Trial, a large multicenter trial of 814 children with no history of stroke or seizure, higher baseline systolic blood pressure was found to be a risk factor for silent cerebral infarction [31]. The prevalence of left ventricular hypertrophy (LVH) has been reported to be as high as 46% in children with SCD but does not correlate with mortality [32]. The finding of high rates of LVH in SCD patients, however, is confounded by the fact that LVH can develop as the result of chronic anemia. Thus, it may be difficult to determine if LVH is related to hypertension, anemia, or both.

As with other forms of CKD, effective control of hypertension, when identified, is likely to slow the progression of CKD in SCN. Optimal blood pressure targets have not yet been defined for SCN but presumably are similar to those of other forms of CKD. While SCD patients can generally be treated with standard approaches, there are disease-specific considerations in the choice of antihypertensive medications. Because dehydration can precipitate vaso-occlusive events and SCD patients often have hyposthenuria, diuretic agents should be avoided in these patients. Angiotensin-converting enzyme inhibitors are frequently utilized in proteinuric renal disease, including SCN. Patients receiving these medications, however, may require additional and more frequent monitoring for increases in serum creatinine and hyperkalemia, particularly during dehydration states or acute pain crises when NSAID use is common.

Tubulointerstitial Disease

Hyposthenuria, Enuresis, and Nocturia

As noted previously, the inability to form a concentrated urine (hyposthenuria) is an early finding in SCD that typically manifests as polyuria. In the BABY HUG study, approximately 20% of infants had hyposthenuria [2, 9]. Of those able to form a concentrated urine, only 38% had a urine osmolality >500 mOsm/kg H_2O. Hyposthenuria can also cause enuresis and nocturia. Enuresis rates in children with SCD ages 5–10 years have been reported to be as high as 40–50% [33]. Enuresis decreases with age, but up to 9% of adults ages 18–20 years will have persistent symptoms. Nocturia is also common in children and adults with SCD, occurring in almost 80% of patients [33]. As a result of defective urinary concentrating ability, patients with SCD may be more susceptible to intravascular volume depletion, which, in turn, can precipitate acute vaso-occlusive events. Maintaining adequate hydration, therefore, is paramount, particularly in settings such as strenuous physical activity and extremes of temperature. For patients with nocturnal enuresis, standard interventions such as avoiding fluids before bedtime may have varying success given the presence of polyuria. Data on the use of desmopressin (DDAVP) for the treatment of nocturnal enuresis in children with SCD are limited to one study of ten SCD patients treated with DDAVP of whom 60% showed improvement or resolution in their enuresis [34].

Hematuria and Papillary Necrosis

Hematuria is a common renal finding in SCD and can occur at any age. Both microscopic hematuria and hemoglobinuria may be present. Papillary necrosis (PN), a more severe manifestation of medullary ischemia, is reported to occur in 15–36% of patients with SCD [35]. Many patients with PN may be asymptomatic. Some, however, can present with acute onset of renal colic, gross hematuria with clots, and acute kidney injury (AKI) with or without urinary tract obstruction. PN can also follow a more protracted course with recurrent episodes of gross hematuria and/or urinary tract infection [12]. The preferred method for diagnosing PN is computerized tomography (CT), which demonstrates the characteristic circumscribed, yet often poorly marginated areas of diminished enhancement in the medullary pyramids, indicating early ischemic changes, as well as clefts originating from the calices [36]. Renal ultrasonography may demonstrate evidence of papillary necrosis on occasion but is generally not as useful for establishing the diagnosis. Due to its self-limiting nature, the management of gross hematuria is usually conservative and limited to maintaining adequate hydration, bed rest, pain relief, and antibiotics if necessary. Additional interventions directed at coagulation and/or anti-fibrinolytic actions studied in small case series include epsilon aminocaproic acid (EACA) and tranexamic acid.

Medullary Carcinoma

Renal medullary carcinoma (RMC) is a rare, aggressive neoplasm that is reported to occur almost exclusively in patients with SCD or SCT [37]. Affected patients generally present with gross hematuria or flank pain. Metastatic disease is often present commonly at diagnosis. The pathogenesis of this malignancy is poorly understood. The prognosis, even with chemotherapy, is extremely poor.

Acute Kidney Injury (AKI)

AKI has been increasingly recognized as an important occurrence in hospitalized children, including those admitted for SCD-related complications. AKI is reported to occur in 8% of SCD children hospitalized for acute chest syndrome [38] and 17% of patients admitted for vaso-occlusive crisis [39]. AKI in SCD patients is associated with prolonged length of stay and increased morbidity and resource utilization [39, 40]. Multiple risk factors for AKI development in SCD have been identified, including older age, history of hypertension, hematuria or CKD, lower hemoglobin level, increased hemolysis, NSAID use, and rhabdomyolysis [4, 39–41]. Angiotensin-converting enzyme inhibitors and the presence of dehydration may also increase AKI risk. The use of HU may decrease the risk of AKI, presumably by improving hemoglobin levels and decreasing the severity of red blood cell sickling [40].

Chronic Kidney Disease (CKD)

Although rates of CKD in patients with SCD vary based on the definition utilized and population studied (including the proportion of more versus less severe SCD genotypes), up to 20% of adults with SCD have CKD [13, 15]. In children, 8–12% have been reported as having CKD Stage 2 or higher [18, 29]. Patients with SCD who develop CKD may manifest complications similar to those of patients with other forms of CKD, such as electrolyte disturbances, edema, hypertension, progressive anemia, and mineral bone disorder. These are generally managed similarly to other forms of CKD. There are, however, several important considerations in CKD management of SCD related to assessments of kidney function, mechanisms and treatment of anemia, and management of ESKD that are highlighted below.

Renal Function Measurements and Imaging Findings

Hyperfiltration, coupled with increased tubular secretion of creatinine, tends to cause low serum creatinine in SCD patients. While hyperfiltration may reflect an actual increase in GFR, increased tubular creatinine secretion would be expected to result in overestimation of actual kidney function. Thus, one of the challenges in accurately determining the degree of renal dysfunction in SCN is identifying a reliable GFR marker that can be followed over time. The BABY HUG trial reported that eGFR (bedside Schwartz formula) correlated only weakly with measured GFR [2]. It should be noted, however, that the study involved infants and young children, the majority of whom had elevated GFRs. Whether the same finding holds true for older children and/or those with decreased GFRs has not been determined. Several adult studies have assessed the utility of eGFR equations compared to measured GFR values and found that the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation showed the best correlation with measured GFRs [42, 43]. Renal biopsy is invasive, has associated risks, and is typically only performed in patients with significant proteinuria.

Renal ultrasonography abnormalities are not uncommon in SCD and may include increased echogenicity, particularly within the medulla, and bilaterally enlarged kidneys [44]. Magnetic resonance imaging (MRI) has also been studied as a potential biomarker for early CKD. MRI with diffusion tensor imaging (DTI) to assess renal microstructure and T2 relaxometry to assess iron deposition has been piloted in a small study [45]. When compared to healthy controls, SCD patients demonstrated MRI changes suggestive of altered medullary microstructure and increased iron deposition. These novel imaging techniques may be able to detect kidney changes before they are clinically apparent (e.g., reduced eGFR).

Anemia in SCD Patients with CKD

In the face of persistent anemia, SCD patients, even those with CKD, typically have elevated erythropoietin (EPO) levels, although not at the levels that would be expected for the degree of anemia. One explanation for the suboptimal EPO levels is the right-shifted hemoglobin-oxygen dissociation curve seen in this patient population. Because sickle hemoglobin has less affinity for oxygen, this translates into better oxygen delivery to target tissues, thereby suppressing the release of EPO. As kidney function declines, this suboptimal EPO production becomes more problematic. As a result, patients with SCD and CKD may require much higher doses of erythropoietin-stimulating agents (ESAs) compared to other patients with CKD to achieve acceptable hemoglobin levels.

Renal Replacement Therapy

Children with SCN who progress to ESKD can be treated with either hemodialysis or peritoneal dialysis using the same selection criteria that are used for pediatric ESKD from other causes. Dialysis management of ESKD in SCN is similar to that of other causes of ESKD [46], with the exception that patients with SCN typically require substantially higher doses of EPO and are more likely to require multiple blood transfusions [47].

Successful kidney transplantation in SCN has been well documented [48, 49], including in a small number of pediatric patients [50]. As is done commonly with other major surgical procedures in SCD patients, preoperative transfusion to decrease the proportion of sickle hemoglobin is recommended for patients undergoing kidney transplantation. In addition, several unique complications have been described. Patients may experience a resumption of frequent vaso-occlusive events, which are presumed to be related to an increase in whole blood viscosity accompanying a higher hemoglobin level [51]. Patients with SCD may benefit from exchange transfusions after kidney transplantation, particularly when hemoglobin levels are elevated.

Prognosis

Despite the multiple mechanisms by which SCD could result in kidney damage and progressive CKD, rates of ESKD in patients with SCD are relatively low. In adults, approximately 2–4% with SCD will progress to ESKD [13, 15]. ESKD in children with SCD is rare. The United States Renal Data Survey (USRDS) database reported that 0.2% of pediatric ESKD cases were attributed to SCD between 2003 and 2012 [52]. Given that patients with SCD are surviving longer due to improved medical care and the increased use of HU as well as newer therapeutics, it is anticipated that ESKD rates will rise. Unfortunately, CKD is an important contributor to decreased survival, accounting for 16–18% of mortality in adults with SCD [4].

Risk Factors for CKD Progression

Hospitalizations for vaso-occlusive crisis have been identified as the most important predictor of progression to ESKD and mortality in patients with SCD [53]. In contrast, in those with biopsy-confirmed glomerular disease, the specific renal pathology (e.g., FSGS vs. MPGN) does not appear to impact overall renal survival [6]. There are several other factors that have been identified as potential risk factors for CKD progression that are discussed below.

Albuminuria

As noted previously, microalbuminuria is a common finding in children and adults with SCD, although it can regress, particularly in children. In adult patients, persistent microalbuminuria has been associated with more rapid decline in eGFR [20]. However, the association of eGFR decline and MA has not been consistently demonstrated in children. In contrast, the prognosis for pediatric and adult patients with SCD and overt proteinuria is poor, with a significant proportion progressing to clinically significant CKD [4, 14].

AKI

AKI, especially severe or repeated AKI episodes, has emerged as an important risk factor for development and progression of CKD in children [54, 55]. As noted above, patients with SCD are at high risk for development of AKI due to multiple factors. While unique associations between AKI and development of CKD in patients with SCD have not been established, it is likely that AKI contributes to CKD in this population as it does in other pediatric and adult populations.

Hemolysis

While chronic vaso-occlusion causes renal damage by multiple mechanisms that likely increase the risk of CKD, chronic hemolysis has also been implicated as a risk factor for development and progression of CKD. In one larger study that examined the relationship between hemolytic markers and hemoglobinuria, both were independently associated with CKD and its progression and were stronger predictors of CKD than indirect hemolytic markers (LDH, reticulocyte count), suggesting that release of cell-free hemoglobin may be an important mediator of renal damage leading to CKD [56].

Genetic Factors

As with other SCD complications, renal dysfunction is more common and more likely to show decline in those patients with SS and Sβ^0thalassemia genotypes [15, 21, 57]. Genetically determined levels of fetal hemoglobin may also impact development and progression of CKD. Patients with the lowest fetal hemoglobin levels are more likely to develop CKD and other SCD complications, whereas those with elevated fetal hemoglobin levels are protected from development of CKD [58].

A growing body of literature has demonstrated the association of apolipoprotein L1 (APOL1) gene risk variants with a significantly increased risk of developing CKD in those of African ancestry [59]. Inheritance of one of the risk alleles (named G1 and G2) is thought to provide protection from trypanosomal disease and, therefore, convey a survival advantage in Africa. Inheritance of two risk alleles, however, is associated with a significantly increased risk of developing albuminuria, CKD, and ESKD [59]. Recent studies in adults suggest that APOL1 risk genotypes are associated with increased risk of CKD in patients with SCD as well [56, 60]. A study of children enrolled in the Sickle Cell Clinical Research and Intervention Program (SCCRIP) study, a lifespan cohort study examining progression of SCD from infancy to adulthood, found that those who carried two APOL1 risk alleles developed albuminuria at a younger age compared to those who had zero or one risk allele [61]. The clinical utility of performing APOL1 genotyping of African Americans in various settings, including kidney transplantation, is the subject of active investigation.

Dialysis and Renal Transplantation Outcomes

Because ESKD due to SCN is relatively rare in the pediatric population, data on dialysis outcomes in children are lacking. Dialysis outcomes for adults with SCN are poor: adults with SCD have higher mortality rates than their non-SCD counterparts [46, 62]. Given the rarity of ESKD in children with SCN, as well as the overall poor outcomes for adult dialysis patients compared to pediatric dialysis patients, it is difficult to know whether children with SCN have similar dialysis-associated mortality.

As with dialysis outcomes, data on kidney transplantation outcomes in children with SCN are limited. One study of 9 children from the NAPRTCS database reported graft survival rates of 89% at 1 year and 71% at 2 years [50]. Of the three patients whose grafts eventually failed, one had acute rejection, one died from an unrelated cause, and one had chronic rejection. Early rejection was frequent and was attributed to inadequate immunosuppression (seen in five of the six episodes). In adult studies, the outcomes of kidney transplantation in SCD patients show considerable variability [63]. In the largest published series to date, age-matched African American kidney transplant recipients with ESKD due to SCN ($n = 82$) were compared to all other causes of ESKD ($n = 22{,}565$) [48]. Rates of delayed graft function and acute rejection were similar in the two groups as was 1-year deceased donor graft survival. However, the 3-year deceased donor graft survival tended to be lower in the SCD group (48% vs. 60%) and the adjusted 3-year risk of graft loss was significantly greater. Adjusted mortality risk in the SCD group was also higher both at 1 year and at 3 years. Two smaller, more recent studies provide additional insight into kidney transplantation outcomes. One study of 36 SCD patients reported postoperative surgical complications in 25% of patients, high rates of CMV and bacterial infections, and a higher mortality rate than non-SCD patients [49]. A study of 34 SCD patients of whom 20 received regular exchange blood transfusions (EBT) also reported worse overall patient and graft survival rates, including higher rejection rates, compared to non-SCD patients. SCD patients who received EBT had lower rates of acute rejection compared to those who did not receive EBT [64]. Thus, although complication rates are higher in both pediatric and adult patients with SCD who undergo renal transplantation compared to patients without SCD, kidney transplantation appears to be the preferred treatment for ESKD in this population, particularly given the significant mortality associated with long-term dialysis therapy.

Sickle Cell Trait

Sickle cell trait (SCT) is defined as inheritance of one copy of the HgbS mutation. It is estimated that SCT affects 1 in 12 African Americans and almost 300 million people worldwide. Although SCT was previously thought to be a benign condition, more recent data suggest that it may be more detrimental than previously appreciated. Hematuria and PN are more common in SCT compared to SCD. This is most likely due to the increased prevalence of SCT as compared to SCD in the general population. It could also be due to the higher hematocrit of people with SCT, which could predispose them to red cell sludging in the kidneys. Medullary renal carcinoma also occurs in patients with SCT. Of additional concern is the growing body of data demonstrating an increased risk for ESKD among those with SCT compared to African Americans who are not carriers. The eGFR of individuals with SCT has been reported to decline significantly faster when compared to healthy reference patients [65]. An observational study of over 1200 patients from 5 US population-based, prospective cohort studies confirmed the high prevalence of SCT among patients with CKD. In that study, SCT was associated with an increased risk of CKD, decline in eGFR, and albuminuria. These associations were independent of APOL1 risk variants and suggest that SCT may be an additional genetic factor contributing to the overall increased risk of CKD in African Americans compared with other populations [66]. In a subsequent study of almost 10,000 self-reported US black patients, the ESKD incidence rate for those with SCT was twice that of non-SCT black patients [67].

Conclusions

Patients with SCD are at risk for the development of numerous renal complications, collectively termed SCN. Given the potential for significant morbidity and mortality associated with SCN, it is imperative that patients be monitored routinely for indications of renal involvement, such as hypertension and albuminuria. Particular attention should be paid to renal function in the context of patients treated with NSAIDs, especially if they are also receiving ACEI therapy. Serum creatinine values and eGFR should be interpreted in the context of known hyperfiltration, and a “normal” eGFR may, in fact, suggest significant decline in function. Continued research is imperative to better elucidate the pathophysiology underlying SCN as well as the contribution of other genetic and/or environmental factors that could be therapeutic targets.

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The Kidney in Type I Diabetes

29

M. Loredana Marcovecchio and
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Abstract

Diabetic kidney disease (DKD) is a serious complication of type 1 diabetes (T1D) and represents a major cause of end stage renal disease (ESRD) and cardiovascular morbidity and mortality. DKD occurs due to interactions between several factors, including hyperglycemia, dyslipidemia, hypertension, oxidative stress, advanced glycated end products, and genetic factors, which induce progressive functional and structural changes in the kidney. Clinically, DKD is characterized by increasing albuminuria, progressive decline in glomerular filtration rate, and elevated blood pressure, which without any treatment progress towards ESRD. Advanced stages of DKD are rare among children and adolescents with T1D, whereas early

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stages, such as hyperfiltration and moderately increased albuminuria, can be common. Several risk factors contribute to the development of DKD, the main being hyperglycemia, high blood pressure, dyslipidemia, diabetes duration, age at diagnosis, female gender, and puberty, along with genetic and environmental factors. Albuminuria, formerly known as microalbuminuria, is considered the earliest clinical manifestation of DKD and is the basis for screening for this complication. Screening for DKD is recommended starting at age 10–12 years and repeated annually. During adolescence, the primary focus of management the risk of DKD is improvement in glycemic control. Targeting additional risk factors, such as hypertension, dyslipidemia, and obesity, is also recommended. When persistent albuminuria is detected, treatment with angiotensin-converting enzyme inhibitors is indicated. There is still a need for new biomarkers to facilitate early detection of subclinical stages of DKD and to support the implementation of intervention strategies, to ultimately reduce the burden of DKD.

Keywords

Type 1 diabetes · Albuminuria · Kidney disease · Youth · Screening · Hyperglycemia · Complications · Treatment · Microvascular

Introduction

Type 1 diabetes (T1D) is a common chronic condition affecting approximately 1.1 million children and adolescents (age <19 years) worldwide, with a growing incidence by 3–4% per year [1]. Individuals with T1D experience increased morbidity and a 2- to 4-fold higher mortality compared to the general population [2, 3]. A young age at the onset of T1D is associated with less favorable outcomes, with life expectancy being reduced by 14–17 years in individuals diagnosed before the age of 10 compared to 9–10 years in those diagnosed after the age of 20 [3]. The long-term health burden of T1D is largely due to the associated microvascular and macrovascular complications [2].

Diabetes kidney disease (DKD), also known as diabetic nephropathy, is one of the most common microvascular complications, affecting around 25–30% individuals with T1D [4, 5]. DKD represents a major cause of end-stage renal disease (ESRD), constituting 10–67% of all cases, and compounds the risks of cardiovascular disease and overall mortality from T1D [4, 5]. In individuals with T1D, DKD is usually a clinical diagnosis based on the presence of albuminuria and/or reduced glomerular filtration rate (GFR), in the absence of signs or symptoms of other primary causes of kidney damage [6, 7]. Other potential forms of kidney disease can occur in the context of diabetes, such as acute kidney injury, often secondary to diabetic ketoacidosis in youth with T1D, and ischemic nephropathy from vascular disease and hypertensive nephrosclerosis mostly in older people with T1D [7].

Natural History of Diabetic Kidney Disease

DKD manifests with the classic triad of increased urinary albumin excretion (albuminuria), renal function decline, and hypertension and is traditionally described as occurring through five sequential stages [8] (Fig. 1). The first stage is generally a "hyperfiltration phase," characterized by increases in GFR, capillary glomerular pressure, and kidney size, without albuminuria or hypertension. The second stage, classically defined as the "silent phase," manifests with progressive increases in urinary albumin excretion that remain within the normal range, and with normal or increased GFR. Structural changes, including basement membrane thickening and mesangial expansion, are observed during this phase [9]. The third stage is characterized by more substantial increases in albumin excretion up to 30–300 mg/day or 20–200 μg/min, referred to as "moderately increased albuminuria" or previously as "microalbuminuria" [8]. With persistent albuminuria, widespread advanced renal structural lesions are observed [9]. GFR during this phase can be increased, normal, or reduced, and hypertension is often present. The fourth

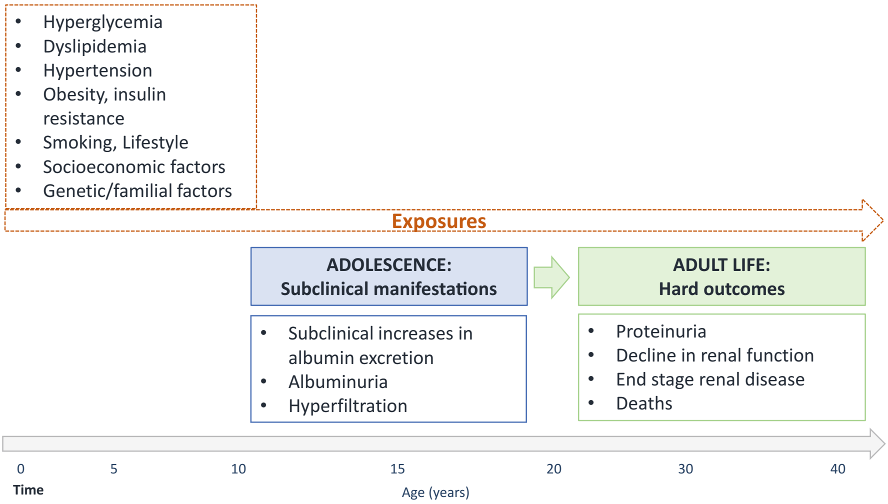
Hyperglycemia
Dyslipidemia
Hypertension
Obesity, insulin resistance
Smoking, Lifestyle
Socioeconomic factors
Genetic/familial factors
Exposures
ADOLESCENCE: Subclinical manifestations
Subclinical increases in albumin excretion
Albuminuria
Hyperfiltration
ADULT LIFE: Hard outcomes
Proteinuria
Decline in renal function
End stage renal disease
Deaths
0
5
10
15
20
30
40
Time
Age (years)

Fig. 1 Risk factors and progression of diabetic kidney disease in youth with type 1 diabetes

stage, occurring 10–15 years after T1D onset, is characterized by overt proteinuria (>300 mg/day) (previously referred as “macroalbuminuria”), decreased GFR (<60 ml/min/1.73m^2), and sustained hypertension. If left untreated, this stage will progress towards renal failure [8]. The fifth and final stage is characterized by uremia and ESRD. At this stage, patients often require renal replacement therapy and/or renal transplantation with simultaneous pancreas transplantation or pancreas-after-kidney transplantation [8].

Over recent decades, there has been a paradigm shift in the natural history of DKD, with the emergence of new concepts. One is the phenomenon of “regression to normoalbuminuria,” which can occur in 30–50% of adults and youth with T1D, and is positively influenced by better glycemic control and lipid and blood pressure profiles [10–12]. This concept has challenged the classical view of microalbuminuria as an irreversible predictor of progression to proteinuria and decline in renal function [11]. Recent data have also shown that in children and adolescents, as in adults, progressive increases in albumin excretion, even remaining within the normal range, can be detected soon after diagnosis and are associated with a higher risk for the development of DKD as well as retinopathy and cardiovascular complications [13]. These findings suggest that albumin excretion should be considered as a predictive biomarker for renal and cardiovascular disease [14]. In addition, data from adults with T1D have shown a “nonalbuminuric DKD” phenotype, whereby early declines in GFR can occur in the absence of albuminuria, which might reflect different renal structural changes [5]. To this point, however, there are no definitive data that this phenotype is found in youth with T1D.

DKD Manifestations in Youth with T1D

Advanced stages of DKD, such as proteinuria, GFR <60 ml/min, or ESRD, are uncommon among youth with T1D [15]. However, early asymptomatic stages, reflecting subclinical renal functional and structural changes, may manifest as early as 1.5–5.0 years after the onset of T1D and are common particularly during adolescence [16, 17] (Fig. 2). These subclinical manifestations include increases in urinary albumin excretion up to the range of albuminuria and increased GFR (hyperfiltration) [16–19]. In adolescents with T1D, rates of hyperfiltration range from 10% to 50% and when present is an independent predictor of albuminuria and later decline in renal function [18–20]. Hyperfiltration, which is often associated with increased renal size, reflects the effect of hyperglycemia on renal hemodynamics, as well as pubertal physical and hormonal changes [18, 19].

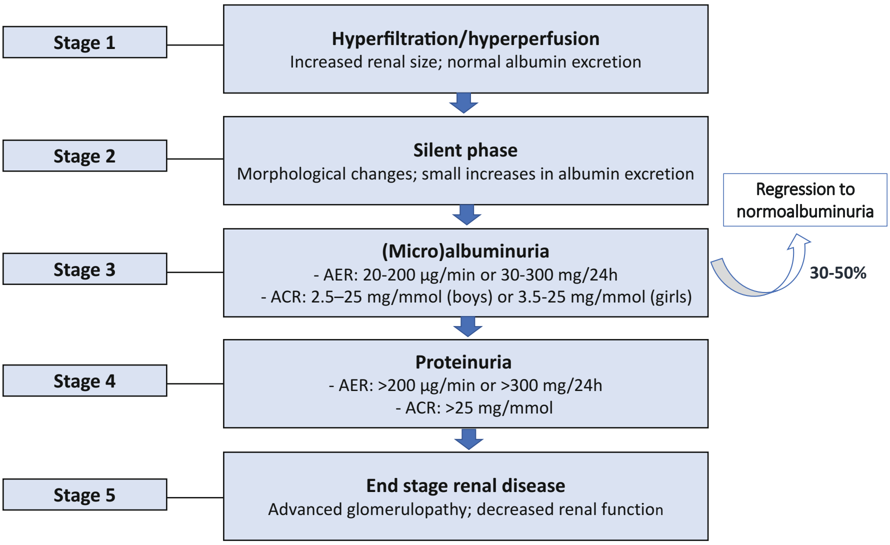
Stage 1
Hyperfiltration/hyperperfusion
Increased renal size; normal albumin excretion
Stage 2
Silent phase
Morphological changes; small increases in albumin excretion
Stage 3
(Micro)albuminuria
- AER: 20-200 µg/min or 30-300 mg/24h
- ACR: 2.5–25 mg/mmol (boys) or 3.5-25 mg/mmol (girls)
Regression to normoalbuminuria
30-50%
Stage 4
Proteinuria
- AER: >200 µg/min or >300 mg/24h
- ACR: >25 mg/mmol
Stage 5
End stage renal disease
Advanced glomerulopathy; decreased renal function

Fig. 2 Natural history of diabetic kidney disease

Albuminuria (or microalbuminuria) is the earliest stage of clinical DKD, and its persistence is predictive of progression to overt proteinuria and cardiovascular sequelae [21]. In people with childhood-onset T1D, microalbuminuria is often detected during puberty, with a cumulative prevalence of around 5–26% at 5–10 years after diagnosis of diabetes, and up to 50% by 19 years after diagnosis of diabetes [10]; in contrast adults with 19 or more years of diabetes have a microalbuminuria prevalence of only 34% [10]. During adolescence, microalbuminuria can be transient or intermittent, with return into the normal range, after 3–10 years, in 30–50% of cases [10, 12]. Although these data are encouraging, transient or intermittent microalbuminuria can become persistent with longer follow-up, likely related to permanent underlining renal structural abnormalities [9, 10]. Therefore, renal function should be repeatedly monitored in any patient developing microalbuminuria. The rate of progression of microalbuminuria to proteinuria appears to be similar between adults and youth with T1D, although proteinuria occurs at an earlier age in younger patients with T1D. In the Oxford Regional Prospective Study cohort, the cumulative prevalence of proteinuria was 13.9% for youth and 14.6% in adults by 19 years after diagnosis of T1D [10].

Pathogenesis

Glomerular basement membrane thickening and mesangial expansion are prominent glomerular lesions in DKD [9, 22]. The pathophysiologic changes in DKD include cellular and extracellular derangements in both glomerular and tubulo-interstitial compartments, including hyperplasia or hypertrophy of various cell types of the glomerulus and tubules, thickening of glomerular and tubular basement membranes, and expansion of the tubulointerstitium and mesangium [9, 22]. Other changes include hyalinization of arterioles and thickening of branches of intrarenal arteries. Foot process effacement and decreased podocyte number and/or density per glomerulus are also common [9, 22]. Diabetic tubulopathy, which may precede or occur concurrently with glomerular changes, is characterized by a variety of structural and functional alterations including tubuloepithelial cell hypertrophy, tubular basement membrane thickening, expansion of the interstitial space with infiltration of various cell types (myofibroblasts and macrophages), epithelial-mesenchymal transition, and glycogen accumulation [9, 22].

The pathogenesis of DKD is incompletely understood, but it likely results from an interplay between several metabolic and hemodynamic factors, which occur as a consequence of hyperglycemia, hypertension, dyslipidemia, genetic predisposing factors, and other environmental exposures [9, 22] (Fig. 2). Hyperglycemia is crucial for the initiation and progression of diabetes-induced renal injury, contributing to abnormal cellular homeostasis and changes in glomerular blood flow and/or vascular permeability, along with tubular and mesangial changes [9, 22]. Hyperglycemia triggers/alters many signaling cascades including activation of diacylglycerol-protein kinase C, polyol and hexosamine pathways; increased oxidative stress, advanced glycation end-products, subclinical inflammation and production of cytokines/growth factors; and dysregulation of the renin-angiotensin system (RAS) [23]. Overproduction of mitochondrial superoxide following hyperglycemia is likely the primary initiating mechanism leading to cellular redox imbalance and oxidative stress [23]. Inflammation, hypoxia, the complement system, and the klotho system also play an important role in the pathogenesis of DKD [22, 23].

Risk Factors

Adolescence is a critical period for the appearance of the first potentially reversible signs of DKD and other diabetes vascular complications [14, 17]. Several risk factors, both modifiable (glycemic control, hypertension, dyslipidemia, lifestyle) and nonmodifiable (diabetes duration, puberty, genes, constitutional factors), have been related to the development of DKD in youth with T1D [17, 24] (Fig. 1). The risk of complications is strongly related to duration of diabetes as DKD tends to reach a peak incidence after 15–20 years [24]. Age at diagnosis is another risk factor, with higher risk of early manifestations of renal disease as well as other vascular complications associated with an early age at the time of diagnosis [10]. Among youth with T1D, biological females have a higher risk of developing microalbuminuria than biological males, which contrasts with the higher risks in adult males [10]. The higher female risk for microalbuminuria may be due to worse glycemic control, higher insulin resistance, increased androgens levels, and lower insulin-like growth factor-1 (IGF-1) levels than in males [17].

Glycemic Control

Hyperglycemia is the major modifiable risk factor for DKD as well as for other vascular complications of T1D. Several observational studies and clinical trials have shown a strong association between glycated hemoglobin (HbA1c) and DKD in youth and adults with T1D [10, 25]. In clinical practice, measurements of HbA1c every 3 months are typically used to assess glycemic control in patients with T1D [26, 27]. Current guidelines from the International Society for Pediatric and Adolescent Diabetes (ISPAD), the American Diabetes Association (ADA), and other organizations recommend a target for HbA1c of <6.5–7% (48–53 mmol/mol), to decrease risk of DKD and other vascular complications [26, 27]. Recent data confirmed that, in youth with T1D, overall DKD risk substantially increases with an HbA1c above 7% (53 mmol/mol) and more advanced stages of DKD, such as proteinuria, are associated with HbA1c $>8.6\%$ (>70 mmol/mol) [28]. Optimal glycemic control is often difficult to achieve during adolescence, with data consistently demonstrating the highest HbA1c levels during this time of life [29] with only 20–30% adolescents achieving recommended HbA1c targets [14]. The increases in HbA1c during adolescence are attributed to poor adherence to diabetes self-management, eating disorders, and/or psychological problems [30]. Additional factors contributing to suboptimal glycemic control are insulin resistance and pubertal hormonal changes [30].

Other Risk Factors

In adolescents with T1D, several factors other than glycemic control, including pubertal growth, hormonal changes, and age-specific psychological issues, can contribute to the development of the early signs of DKD [17, 30]. Puberty is characterized by rapid renal growth and glomerular hyperfiltration, both of which exacerbates DKD risk, independently of glycemic control [19]. These changes in kidney structure and function may be linked to increased circulating growth hormone and decreased IGF-1 levels, which are both associated with a higher risk of microalbuminuria [17]. Cardiometabolic risk factors such as obesity, hypertension, dyslipidemia are common among adolescents with T1D and contribute to complication risks [31]. Abnormalities in blood pressure, including reduced nocturnal dipping and increased diastolic blood pressure, predict for adolescents who later develop microalbuminuria [26]. Youth with T1D also have a high prevalence of dyslipidemia, characterized by both qualitative and quantitative abnormalities in lipids and/or lipoproteins, both of which are associated with rates of urinary albumin excretion [26]. Obesity is common among youth with T1D (9–20%), and a high BMI is associated with increased rates of DKD [31, 32]. Lifestyle factors, such as smoking, alcohol, sedentary lifestyle, diet, and stress, can also increase risks of complications [31].

Emerging data also show associations between socioeconomic factors, such as lower education levels and household income, and greater risks of adverse renal outcomes [33].

Genetic factors also likely contribute to the risk of DKD, as studies show a 2–4 fold higher risk of developing DKD for a proband with a sibling that has DKD [34]. In addition, a family history of insulin resistance, hypertension, dyslipidemia, and/or cardiovascular disease is more common among adolescents with microalbuminuria than those without microalbuminuria [35].

Screening and Diagnosis

DKD as well as the other vascular complications of T1D are often asymptomatic during early stages, and once symptoms develop, disease progression may be harder to reverse [14, 26]. Therefore, identification of subjects at risk and screening for subclinical signs of complications is essential for the implementation of timely preventive and therapeutic strategies. International guidelines recommend screening for DKD starting at ages between 10 and 12 years. These recommendations are based on evidence suggesting that complications are rare in younger children [26]. Based on the ISPAD guidelines, screening should start at age 11 years with 2–5 years diabetes duration and repeated annually, with potential variation in the frequency based on the individual's risk profile [26].

Screening for DKD is focused on detection of increased albuminuria, which can be assessed with 24-hour or timed urine collections or as albumin-creatinine ratio (ACR) on a spot urine sample [26]. Assessing ACR in a spot urinary sample is the most common method used in clinical practice. First morning voided urine is preferable due to diurnal variations in albumin excretion and postural effects; however, if this is not possible, uniformity of timing for serial collections in the same individual should be employed [26]. When using ACR, values between 2.5 and 25 mg/mmol in males and 3.5 and 25 mg/mmol in females denote moderately increased albuminuria or microalbuminuria (different reference ranges are due to sex-related differences in creatinine excretion) [26]. On 24-hour urine samples, 20–200 μg/min or 30–300 mg/day represents microalbuminuria. Values above these limits for spot or 24 hour samples are diagnostic of proteinuria/macroalbuminuria [26].

Exercise, infections, fever, marked hyperglycemia, other types of kidney disease, and/or systemic inflammatory diseases can influence albumin excretion, and therefore, these factors need to be excluded when interpreting elevated albumin excretion. Confirmation of persistent albuminuria is based on high levels on serial samples (at least 2 abnormal values among 3 samples collected over a period of 3–6 months), since there is substantial within-subject variation in urinary albumin excretion. Regular longitudinal follow-up of albumin excretion is also important to identify patients with progressively small increases of urinary albumin excretion within the normal range, which might presage the development of microalbuminuria [14, 26].

Although urinary albumin excretion is generally the cornerstone for the diagnosis of DKD, identifying alterations in GFR may be important during the early and late stages of DKD. Presently, routine assessment of GFR is not typically included in pediatric guidelines [26]. In clinical research and practice, GFR is typically estimated by equations using endogenous filtration markers (serum creatinine and/or cystatin C) often adjusted for age, sex, and height. While many formulas using these endogenous filtration markers have been proposed in children and adolescents, no single equation has been specifically developed or validated in youth with T1D [20]. Most formulas to estimate eGFR are less accurate in the hyperfiltration range and therefore are not optimal to use in adolescents with T1D [20]. Improved methods to easily and accurately measure GFR as well as changes in renal function in both the normal and hyperfiltration range are therefore needed. Clinicians must also evaluate youth with T1D with apparent new renal disease for possible nondiabetic causes, as part of the diagnostic workout. An active urinary sediment (e.g., hematuria), rapidly increasing albuminuria or nephrotic syndrome, and/or a rapidly decreasing GFR suggests alternative and/or additional causes of kidney disease. For patients with these features, referral to a nephrologist for further workup, including the possibility of kidney biopsy, should be considered [6].

New Biomarkers

There is a growing interest in identifying new biomarkers which could replace GFR and ACR or improve their predictive value for the early identification of DKD and prediction of its progression [36–38]. Recent advancements in proteomics, metabolomics, and other "omics" have identified new potential biomarkers for DKD [38]. Newer DKD biomarkers identified to date include those in the following categories: glomerular and renal tubular biomarkers, cardiovascular biomarkers and inflammation-, oxidative stress-, and fibrosis-related biomarkers. A renal biomarker which has been largely investigated is cystatin C, a plasma protein produced by all nucleated cells, freely filtered by glomeruli and completely metabolized by proximal renal tubules, which has been associated with ESRD [36]. Several inflammatory biomarkers have been investigated in relation to DKD, including tumor necrosis factor receptors, orosomucoid, fibroblast growth factor-23, cytokines (IL-6, IL-10, INF-gamma, TNF-α), and growth/differentiation factor-*15*, YKL-40 [22, 36]. Proximal tubular proteins, such as urinary Kidney Injury Molecule-1, Neutrophil gelatinase-associated lipocalin, and liver-type fatty acid-binding protein, have been associated with a faster decline in GFR in individuals with T1D [22, 36]. Studies have also identified that cardiovascular disease biomarkers, such as high-sensitivity troponin T and N-terminal pro-B-type natriuretic peptide, can predict decline in renal function in T1D [22, 36]. Uric acid, a product of purine metabolism, has recently shown promise in predicting renal function impairment in individuals with T1D [36]. Urinary proteomics, a promising tool to identify biomarkers of DKD, has identified CDK273, a panel of 273 urinary biomarkers, that is associated with progression of albuminuria and loss of renal function in adults with diabetes [39]. Metabolomics has also been applied in the field of DKD and unveiled several metabolites including products of lipid metabolism, branched chain and aromatic amino acids, and citric acid cycle metabolites related to mitochondrial dysfunction that may predict for poor outcomes [22, 36]. To date, however, none of these promising new biomarkers have been integrated into clinical practice.

Prevention and Treatment

Glycemic Control

During adolescence, the primary focus for DKD risk management is on improvements in glycemic control [26, 27]. The benefits of achieving HbA1c targets (HbA1c <6.5–7.0% or <48–53 mmol/mol) to prevent vascular complications is supported by a large body of evidence, starting with the landmark Diabetes Control and Complications Trial and including more recent studies [25]. Multiple daily insulin injections and continuous subcutaneous insulin infusion (CSII) are the main treatment modalities in youth with T1D. Over the last several years, there have been significant advances in technology for the management of T1D, with the introduction of CSII and continuous glucose monitoring (CGM); more recently, automated insulin delivery systems or close loop systems (artificial pancreas), which combine real-time CGM with CSII and a control algorithm to direct automated insulin administration, have contributed to better glycemic control [40]. Use of CGM allows the evaluation of glucose variability, which has emerged as another potential target to prevent diabetes complications in adults with type 2 DM (to date scant data are available for T1D, particularly for the pediatric population). In addition, CGM data have shown the importance of time in range (TIR), defined as the percentage of time when glucose values are within an acceptable range (70–180 mg/dl or 3.9–10.0 mmol/l) [41] Recent pilot data have indicated that patients have a 40% increased risk of DKD for each 10 percentage points lower TIR [42].

Additional Treatment Strategies

In adults with T1D, the presence of microalbuminuria is a general indication for intervention with angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and/or statins. These recommendations are based on strong evidence that ACEIs decrease the progression of DKD and increase the likelihood of regression to normoalbuminuria. However, ACEIs have not been shown to prevent DKD [6]. Statin treatment may reduce rates of proteinuria and progression of chronic kidney disease in adults with T1D [6]. In adolescents with T1D, there is no definitive consensus on the use of ACEIs and/or statins when microalbuminuria is detected. Based on current guidelines, treatment with ACEIs is indicated only for those adolescents with persistent albuminuria, based on data from a few small studies [26, 27]. ACEIs are typically the first line treatment for persistent microalbuminuria, with ARBs being recommended for patients showing intolerance to ACEIs. A recent large clinical trial, the Adolescent Type 1 Diabetes cardiorenal Intervention Trial (AdDIT), assessed the effect of ACEIs for the prevention of DKD in adolescents that had ACR in the upper tertile of the normal range [43]. The trial did not show a clear effect of ACEIs in reducing ACR levels over a 2- to 4-year treatment period, although there was a trend for fewer new cases of microalbuminuria [43]. At a minimum, the trial provided reassuring data on the safety of ACEIs in this age group. ACEI side effects were mostly hypotension requiring dose reduction, whereas few patients developed cough [43]. At present clinicians should assess patients on an individual basis; in those with increasing ACR and/or other concomitant risk factors, ACEIs could be used given the good safety data [14]. If females are prescribed ACEIs/ARBs, it is important that they are counseled on the teratogenic risks associated with pregnancy [26, 27].

Guidelines recommend lifestyle interventions (diet and physical activity) as first steps to control dyslipidemia and hypertension [26, 27]. Initial treatment of elevated blood pressure includes dietary modification and increased exercise, but if targets are not reached within 3–6 months, pharmacologic interventions with antihypertensive drugs should be considered [26, 27]. ACEIs are the first line treatment when albuminuria is also detected. Similarly, if patients have dyslipidemia, a specific nutrition plan should be implemented first, followed by the introduction of a statin if LDL-cholesterol targets (<3.4 mmol/l or <130 mg/dL) are not reached. The AdDIT trial showed the beneficial effect of statins in reducing lipid levels and also provided important data on the safety of this class of medications in youth with T1D [43]. Raising awareness about the adverse effect of smoking and alcohol among youth with T1D and implementing strategies to prevent these habits are other key management strategies. Lifestyle interventions to prevent or reduce obesity rates are also recommended [26, 27].

Other Interventions

Several additional treatments have been investigated to better manage DKD and other vascular complications, mainly in adults with T1D, although data are often limited in younger patients. Sodium-glucose co-transporter 2 inhibitors (SGLT2i) have shown large benefits on cardiovascular and renal outcomes in adults with T1D. SGLT-2i may ultimately be beneficial in adolescents with T1D due to their high rates of hyperfiltration and evidence suggesting relatively low RAS activity with predominantly afferent dilation rather than efferent constriction in adolescents with T1D [44]. However, the risk for euglycemic ketoacidosis is a potential limiting adverse event for the use of these medications. There has also been interest in the potential role of uric acid in the pathogenesis of DKD and as a potential target for treatment. However, a recent trial did not show any clinically meaningful benefits of serum urate reduction with allopurinol on kidney outcomes in adults with T1D [45].

Conclusions

As the incidence of T1D continues to rise, particularly in children and adolescents, the burden of DKD will also increase and negatively influence the prognosis of young people with the disease. Therefore, early detection of risk factors and signs of DKD is of paramount importance to facilitate the early implementation of intervention strategies, which could reduce DKD development and/or progression. Further investigation to clarify etiological factors, both genetic and environmental, implicated in the pathogenesis of DKD, and the identification of new biomarkers, could better identify individuals at risk for developing DKD and guide more effective preventive and therapeutic strategies.

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The Kidney in Metabolic Syndrome

30

Stella Stabouli and Mark Mitsnefes

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Abstract

Increasing epidemiologic evidence shows that obesity and metabolic syndrome (MS) are associated with increased risk of developing chronic kidney disease (CKD). Recent advances in the pathophysiology of obesity-related kidney disease indicate that hemodynamic factors, chronic inflammation, and abnormal lipid metabolism contribute to kidney injury. However, all components of MS, hypertension, atherogenic dyslipidemia, and type 2 diabetes mellitus (T2DM) may induce kidney damage. Children with severe obesity have increased prevalence of early kidney abnormalities, including hyperfiltration, albuminuria, and elevated early urinary biomarkers of kidney injury. Treatment of obesity through lifestyle modification is the mainstream of management of MS. If successful, it is typically associated with the reduction of all other MS cardiometabolic risk components. Pharmacological treatment with angiotensin converting enzyme inhibitors and bariatric surgery in case of severe obesity could provide long-term benefit by slowing the progression to CKD. Novel renoprotective agents, such as GLP-1 analogues and SGLT2 inhibitors need to be evaluated for clinical use in children and adolescents with MS.

Keywords

Metabolic syndrome · Obesity · Hyperfiltration · Chronic kidney disease · Albuminuria · Bariatric surgery · Children and adolescents

Introduction

The obesity epidemic remains one of the most challenging health issues affecting children and adolescents at alarming rates. In 2016, the prevalence of obesity was 6% in girls and 8% in boys compared to less than 1% in both sexes in 1975. According to World Health Organization (WHO), in 2016 over 340 million children and adolescents were overweight; data from 2019 show 38 million overweight or obese children under the age of 5 years [1]. Cardiometabolic risk factors accompany overweight and obesity in children and adolescents with increased prevalence and severity in those at higher classification stages of obesity [2]. As body mass index (BMI) increases, the risk for cardiometabolic multimorbidity [3], and therefore the risk for metabolic syndrome (MS), defined as a constellation of three or more cardiometabolic risk factors in an overweight or obese individual, also increases. Since first described by Raven in the 1980s, the diagnosis of MS has been widely questioned in the literature. Definitions of MS in children have been published by many scientific organizations; however, a consensus to define MS has not been reached making it difficult to attain conclusive data on health outcomes in the pediatric population. Nevertheless, MS is an acquired, noncommunicable, possibly preventable disease. While screening, diagnosis and treatment of MS is mainly targeted in prevention of future cardiovascular disease [4]; it has been increasingly recognized that obesity and MS are significant risk factors for chronic kidney disease (CKD) [5], an association that seems to have its origins in childhood [6]. Thus, screening and management of MS in childhood may also alter long-term kidney outcomes.

Definitions of MS in Children

A uniform definition for MS in children and adolescents is lacking, and more than 40 definitions have been used in pediatric studies [7]. The most widely accepted definition of MS in children and adolescents has been proposed by the International Diabetes Foundation and includes central obesity as a necessary criterion with an addition of two or more cardiometabolic risk factors (Table 1) [8]. This definition focuses on visceral adiposity as the main drive between insulin resistance and cardiometabolic risk, such as hypertension and diabetes. Another widely used definition of MS in children developed by the National Heart, Lung, and Blood Institute does not include obesity as a necessary criterion [9]. None of the definitions focus on the severity of obesity which seems to be a major determinant of cardiometabolic clustering [2, 3].

Effect of Cardiometabolic Parameters on the Kidney

Mechanisms of Kidney Damage

Obesity-induced kidney damage has been extensively highlighted in the recent years. However, all components of MS, hypertension, atherogenic

Table 1 Definitions of metabolic syndrome in children and adolescents [8, 9]

	International Diabetes Federation 2007	Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents 2011
Definition	WC ≥90th plus any 2 ≥ criteria	Any ≥3 of 5 criteria
Obesity	**6- <10 years:** WC ≥ 90th pc **10- < 16 years:** WC ≥ 90th pc or adult cut off if lower **>16 years:** WC ≥ 94 cm in Caucasian males or ≥ 80 cm in females or other ethnic specific values	BMI ≥ 85th pc or WC ≥ 90th pc
High triglycerides	**6- < 10 years:** NA **10- < 16 years:** ≥ 150 mg/dl >**16 years:** ≥ 150 mg/dl or treatment for high triglycerides	≥75 mg/dl if 0–9 years ≥90 mg/dl if ≥10 years
Low HDL	**6- < 10 years:** NA **10- < 16 years**: <40 mg/dl **>16 years:** <40 mg/dl in males <50 mg/dl in females or treatment for low HDL	≤ 40 mg/dl
Non-HDL	NA	≥ 120 mg/dl
High blood pressure	**6- < 10 years:** NA **10- < 16 years:** ≥130/85 mmHg **>16 years:** ≥130/85 mmHg or diagnosis of hypertension	≥ 90th age, sex, and height percentile
Glucose intolerance	**6- < 10 years:** NA **>10 years:** Fasting glucose ≥100 mg/dl or known type 2 diabetes mellitus	Fasting glucose ≥100 mg/dl

Abbreviations: BMI body mass index, WC waist circumference, HDL low density lipoprotein, NA not available

dyslipidemia, and type 2 diabetes mellitus (T2DM), may also individually induce kidney damage (Fig. 1). Metabolically healthy obese individuals (i.e., obesity in the absence of any cardiometabolic risk factors) present an at-risk population for future incident CKD, but their odds of having CKD is lower compared to metabolically unhealthy obese patients [6]. The number of clustering cardiometabolic risk factors is associated with higher odds for incidence or progression of CKD [10]. Of note, the duration of exposure to metabolic abnormalities that leads to irreversible kidney damage is not well defined by current evidence.

In obesity, hemodynamic changes and overproduction of adipokines and growth factors cause glomerulomegaly and ultimately focal-segmental glomerulosclerosis (FSGS) [11]. Hypertension causes kidney injury mainly through increase in intraglomerular pressure [12]. Atherogenic dyslipidemia, with low high-density lipoprotein (HDL) and high levels of triglycerides, induces endothelial damage in the blood vessels of the kidney and lipotoxic injury in glomerular, mesangial, and tubular cells [11]. Hyperglycemia-induced kidney damage involves several pathogenic pathways including increased formation of advanced glycation end-products, activation of protein kinase C isoforms both leading to oxidative stress and accumulation of inflammatory elements, as well as increased production of transforming growth factor β1 (TGF-β1) exerting profibrotic actions and inducing increased production of extracellular matrix (ECM) by mesangial cells [13]. Increased ECM accumulation causes thickening of the glomerular and tubular basement membranes, mesangial expansion, sclerosis, and tubulointerstitial fibrosis that are characteristic features of diabetic kidney damage [14]. Insulin resistance is associated with all components of MS and has a key role in the pathogenesis of kidney damage by inducing glomerular hyperfiltration and increased vascular permeability [14]. In addition, adipocytokine dysregulation and low-grade

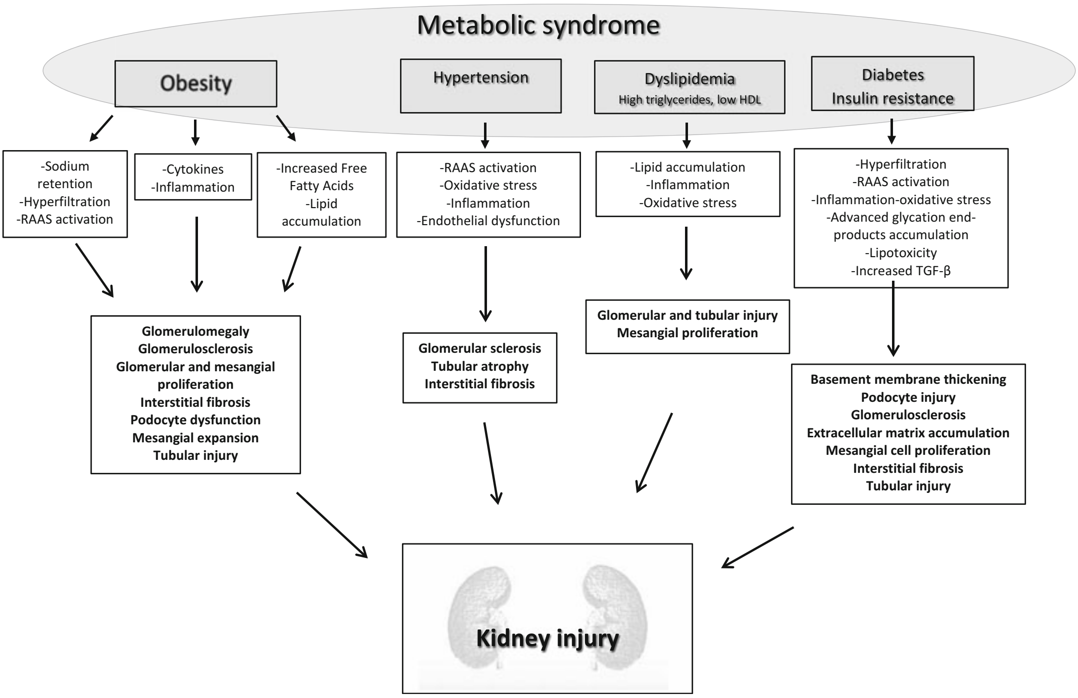

Metabolic syndrome
Obesity
Hypertension
Dyslipidemia
High triglycerides, low HDL
Diabetes
Insulin resistance
-Sodium retention
-Hyperfiltration
-RAAS activation
-Cytokines
-Inflammation
-Increased Free Fatty Acids
-Lipid accumulation
-RAAS activation
-Oxidative stress
-Inflammation
-Endothelial dysfunction
-Lipid accumulation
-Inflammation
-Oxidative stress
-Hyperfiltration
-RAAS activation
-Inflammation-oxidative stress
-Advanced glycation end-products accumulation
-Lipotoxicity
-Increased TGF-β
Glomerulomegaly
Glomerulosclerosis
Glomerular and mesangial proliferation
Interstitial fibrosis
Podocyte dysfunction
Mesangial expansion
Tubular injury
Glomerular sclerosis
Tubular atrophy
Interstitial fibrosis
Glomerular and tubular injury
Mesangial proliferation
Basement membrane thickening
Podocyte injury
Glomerulosclerosis
Extracellular matrix accumulation
Mesangial cell proliferation
Interstitial fibrosis
Tubular injury
Kidney injury

Fig. 1 Pathophysiologic mechanisms of MS-related kidney damage

inflammation, which mediate endothelial dysfunction and vascular damage, have been proposed as additional pathways involved in the modulation of kidney function in the presence of insulin resistance. Insulin resistance may also act at the level of the podocyte, regulating podocyte apoptosis, and thus, leading to glomerular basement membrane thickening, glomerulosclerosis, and albuminuria [13]. Finally, both in obesity and diabetes, increased ultrafiltration flow into Bowman's space heightens shear stress on the podocyte. This mechanical stress leads to an increase in glomerular basement membrane length and to podocyte hypertrophy.

Obesity-Induced Glomerulopathy

Obesity-induced glomerulopathy represents a distinct clinical entity characterized by proteinuria and progressive decline of renal function [11]. The primary histologic features might include relatively few FSGS lesions, glomerulomegaly, due to glomerular hyalinosis and fibrosis, as well as lipid accumulation in the glomeruli and adhesion to Bowman's capsule [11]. Glomerulomegaly is the defining feature presenting in 100% of cases. Perihilar glomerular sclerosis is common in obesity-induced glomerulopathy and might associate with greater ultrafiltration pressure [11]. As the glomeruli enlarge, the numerical density of podocytes decreases due to lower podocyte ability of adaptive hypertrophy eventually resulting in podocyte detachment and local denudation of the glomerular basement membrane and subsequently to FSGS development [11].

Early studies on adults with obesity-related kidney disease reported nephrotic-range proteinuria or decreased renal function with extensive histologic injury, glomerulosclerosis, and chronic tubulo-interstitial damage [15]. The outcome for these patients was poor, with up to 50% progressing to end-stage kidney disease (ESKD) within 10 years of diagnosis [15]. Of note, even patients with nephrotic range proteinuria rarely present with clinical features of nephrotic syndrome [11]. Isolated subnephrotic proteinuria is found in the majority of patients with obesity-induced glomerulopathy.

Importantly, pathological glomerular lesions may be found in extremely obese patients without clinical renal manifestations [16].

During the early phases of obesity, prior to loss of nephron function due to glomerular injury, obese individuals present with increased sodium reabsorption. This may be compensated for by renal vasodilation, increased glomerular filtration rate (GFR), and increased filtered amount of water and electrolytes as hypertension is compensated for by the pressure natriuresis. Increased intrarenal pressure caused by fat accumulation in the renal medulla compresses the loops of Henle and vasa recta, causes activation of the renin-angiotensin-aldosterone system (RAAS), and increases sodium reabsorption in both proximal and distal tubules [12]. Detection of the decreased amount of sodium, reaching the distal tubular cells, by the macula densa, leads to a rise in renin secretion, via tubuloglomerular feedback. Moreover, adipose cells may represent a major site where all components of the RAAS are formed. Renin, angiotensin II, angiotensinogen, and angiotensin II-receptors are found in abundance in adipose mass suggesting that a local tissue angiotensin system is present at the adipocyte level [12]. Overexpression of angiotensin II induces increase in proliferative factors such as TGF-β and plasminogen activator inhibitor. The tissue and the circulating RAAS components are in a state of constant interaction. RAAS activation might also contribute to hyperfiltration as angiotensin II and aldosterone have a greater vasoconstrictive effect on the efferent rather than the afferent arterioles. This results in imbalance between afferent and efferent renal vascular tones, and increasing transcapillary hydraulic pressure difference and therefore the GFR [11].

Adipokines secreted by the adipose tissue are shown to mediate kidney injury through chronic inflammation. Decreased levels of the adiponectin and high levels of leptin in obesity are associated with increased insulin resistance and inflammation with free fatty acid (FFA) accumulation. Adiponectin may prevent glomerular and interstitial damage, and low adiponectin in obese individuals has been associated with progression of kidney disease. Leptin actions through the hypothalamic neuronal systems include a decrease in food consumption and upregulation of thermogenesis and energy expenditure, via stimulation of sympathetic activity [12]. Leptin may also directly stimulate glomerular cell proliferation and mesangial cell hypertrophy [11].

Lipotoxicity due to increased fatty acid synthesis and defective free fatty acid (FFA) oxidation with increased accumulation of lipids and lipoproteins in glomerular and interstitial cells is another metabolic dysregulation of obesity that induces kidney damage [17]. Local overproduction of inflammatory cytokines and growth factors by free fatty acid accumulation and mitochondrial damage results in ongoing cellular injury [17].

Physical compression of both kidneys appears to generate from accumulation of adipose tissue around the organs emphasizing the crucial role of visceral obesity in the development of renal disease [12]. Deposition of ECM throughout the renal medulla is greatly expanded, and the tissue surrounding the ducts of Bellini at the vascular pole tends to prolapse. Increased numbers of interstitial cells and an increase in material rich in lipids and proteoglycans compress the renal parenchyma toward the pole of the kidney resulting in round-shaped, enlarged kidneys in obese subjects [18].

Epidemiological Evidence Linking Childhood MS to Kidney Outcomes

The main MS-related clinical outcomes that affect the kidney include intermediate endpoints (e.g., hyperfiltration, microalbuminuria, and kidney stones) as well as hard endpoints (e.g., ESKD). Still, the clinical course of kidney disease in childhood and adolescence is not extensively studied. The duration of MS might be a key factor for the risk of CKD [19].

Both renal hyperfiltration and hypofiltration have been reported in patients with MS [20, 21]. Although hyperfiltration may be seen at early stage and hypofiltration at late stage of kidney damage, the distinct components of MS may also have different impacts on the kidney. A study including 205,382 adults, aged 40–74 years, showed that hyperfiltration was associated with prehypertension and prediabetes, while hypofiltration was associated with dyslipidemia, abdominal obesity, overt hypertension, and overt diabetes [20].

Glomerular hyperfiltration, an early marker of obesity- or MS-related kidney disease may be evident before the appearance of histological changes and caused by the increased metabolic demands of obese individuals. Younger age, high-normal or elevated BP, and prediabetes may characterize obese individuals with hyperfiltration [20]. This may also be the case in children and adolescents [21–23]. A hyperfiltration state may be present, in part, due to intrarenal vasodilation induced by insulin resistance in a compensatory response to overcome the increased tubular reabsorption of sodium in obese individuals. In adolescents, evidence from the NHANES shows that hyperfiltration is associated with hypertriglyceridemia and insulin resistance even after accounting for BMI [24]. Higher GFR has been reported in the majority of population studies in obese children compared to those with normal weight [21–23, 25]. In a case-control study of 596 children and adolescents with overweight/obesity recruited from outpatient clinics in a single center in Italy, the prevalence of hyperfiltration and CKD stage 2 was higher compared to controls [21]. Reduced GFR has been reported in obese children in a population-based survey from Turkey. However, there was no statistical difference in the prevalence of CKD stages 2–5 when compared to children without CKD [26].

A meta-analysis on 57 studies in the general adult population showed a twofold higher risk for albuminuria and proteinuria in those with MS [27]. This association was independent of diabetes and more pronounced in men. The impact of MS was greater than each component separately with the number of clustering factors presenting a dose-response association for albuminuria risk. Obesity, the main MS component, has been consistently reported as an independent risk factor for microalbuminuria and/or proteinuria in several pediatric studies [21, 28–30]. In obese adolescents, increased urinary albumin-to-creatinine ratio (ACR) has been associated with fasting hyperinsulinemia, an impaired glucose tolerance test and hypercholesterolemia [31]. The prevalence of microalbuminuria in obese children has been reported from 0.3% in obese adolescents in the NHANES up to 14.7% in obese children from pediatric obesity clinics [23, 28–30, 32]. In the majority of studies, higher BMI was independently associated with both increased GFR and ACR. In one study, the main determinants of ACR were high serum insulin and triglyceride levels [32].

Adult-onset CKD is attributed mainly to diabetes and hypertension, and its progression could be accelerated in the presence of obesity. Childhood onset of these chronic conditions adds additional exposure years and could increase the risk for CKD in adulthood [25]. Of note, a preexisting state of a reduced number of nephrons as a result of perinatal, congenital, or unknown environmental and lifestyle factors or genetic predisposition cannot be excluded [25].

The effect of other MS components on the kidney in children without CKD has been reported in a few cohort and cross-sectional studies. Large population-based cohort studies from the Israeli ESKD registry suggest a role for cardiometabolic risk factors in adolescence on the incidence of ESKD in adulthood. In over two million asymptomatic healthy adolescents followed for a median period of 16.8 years, those with prehypertension had a 32% increased risk for subsequent ESKD, compared to adolescents with optimal BP [33]. In another study from the same registry including 1.2 million adolescents without a diagnosis of kidney disease, BMI at the age of 17 years was associated with increased incidence of future ESKD. Overweight and obesity were associated with 3 and 6.89 times (respectively) increased risk for all-cause-treated ESKD during 25 years of follow-up [5]. Similarly, in a case-control study from the Swedish National Population Register, being overweight at age 20 or obese at any later time point and without diabetes or hypertension was linked to an increased risk for CKD, suggesting causal pathways by elevated BMI [34].

MS and diabetes are considered among the major risk factors for uric acid nephrolithiasis [35]. Insulin resistance is inversely associated with renal ammonium synthesis and urinary pH, which increases the risk of developing uric acid nephrolithiasis, oxalate calcium stones, or a combination of both [35]. There are limited data in children and adolescents with MS. Increased serum uric acid levels have been reported in obese children with kidney stones [36].

MS-Related Kidney Outcomes in Special Populations

Children with Low Birth Weight and/or Prematurity

Low birth weight for gestational age and prematurity have been associated with reduced nephron numbers in humans [37]. Developmentally, programmed nephron deficit might contribute to the increased susceptibility to hypertension and renal disorders that are more prevalent among populations with a trend toward low birth weight, especially following exposure to subsequent renal stresses such as hypertension or diabetes. In children and adolescents born preterm and/or with low birth weight, subsequent overnutrition and obesity might act synergistically to augment the effects of developmental programming on the risk of kidney disease later in life. Based on the findings from a histopathological study in children with obesity-related nondiabetic proteinuric kidney disease, preterm patients were more likely to have reduced renal mass assessed by renal ultrasound, and obese preterm children had a twofold higher risk of progression to CKD stage 5 with a median renal survival of 15 years compared to 23 years in obese term children [38]. The synergistic effect of obesity on individuals with reduced nephron mass has also been shown in patients with unilateral renal agenesis or a severe reduction in renal mass as a result of medical or surgical intervention [39]. BMI at presentation was one of the factors significantly associated with the development of proteinuria and loss of renal function in these patients.

Preexisting-CKD and Kidney Transplantation

The detrimental effect of MS on progression of CKD has been well described in adults. The effect of MS on children with preexisting CKD is an emerging field. In the Chronic Kidney Disease in Childhood (CKiD) study, a multicenter, prospective cohort study in North America, among 472 children with estimated GFR 30–90 mL/min/1.73m^2 the prevalence of MS was 15.1% (40% among overweight patients and 60% among obese) [40]. MS was associated with twofold greater odds of an annualized eGFR decline of >10% [40]. The prevalence of MS is high in pediatric kidney transplant recipients ranging from 18% to 40% [41–43]. It has been reported that the likelihood of posttransplant MS is 16-fold higher compared to general pediatric population [44]. Steroid use is associated with the incidence or persistence of MS after kidney transplantation with prevalence up to 68% in those on steroids compared to 15–20% in steroid withdrawal groups [43, 45]. A faster decline of allograft function was observed in pediatric patients with MS at 1 and 1.5 years after kidney transplantation [45].

Most of the studies examining the effect of posttransplant cardiometabolic risk factors in children and adolescents on allograft function have mainly focused on hypertension and obesity, while the role of dyslipidemia and diabetes is less well studied. Hypertension was reported to associate with poorer graft survival in the long term [46], but the high BP detrimental effect on the graft is usually evident early after transplantation [47]. Obesity seems to have an adverse impact on graft survival [42, 48, 49], especially in cases with rapid weight gain after transplantation, although some studies showed neutral effect [50, 51]. Of note, all studies assessing the role of obesity postkidney transplant had a relatively short follow-up. Pretransplant excess weight has been associated with increased risk for posttransplant obesity [52], cardiometabolic risk factors, and worse graft survival [53]. Finally, large annual increase in BMI z scores has been associated with increased risk of death (adjusted hazard ratio 1.44, 95% confidence intervals 1.07–1.92) in a retrospective cohort of 1,182 pediatric dialysis and transplant patients [54].

Glomerular Disease

The prevalence of MS components is increased in children with glomerular diseases [55]. In a study from the CKiD cohort, including 586 children with an estimated GFR between 30 and 90 ml/min/1.73m^2, 15% were obese, 44% had dyslipidemia, 46% had hypertension, and 21% had abnormal glucose metabolism. Thirteen percent had three or more cardiometabolic risk factors and thus, by definition, having MS [56]. Glomerular disease was associated with 1.96 higher odds of cardiometabolic risk factor clustering. Data from a retrospective study in 446 pediatric patients with Henoch-Schoenlein Purpura Nephritis showed that obesity was associated with a 3.27 increased risk of progression to ESKD [57]. In adults with IgA nephropathy, obesity was associated with the presence of interstitial fibrosis and faster GFR decline [75, 76]. However, some studies question independent effect of obesity on progression of IgA nephropathy noting that the effect of obesity could be mediated by the higher cluster of cardiometabolic risk factors rather than obesity per se [58].

Renal Complications of Type 2 Diabetes Mellitus in Children and Adolescents

Two large studies of children and adolescents with T2DM conducted over last the two decades have evaluated the development of diabetic kidney disease (DKD). The SEARCH for Diabetes in Youth (SEARCH) study is an observational multicenter study focusing on physician-diagnosed diabetes (both type 1 and 2) in individuals less than 20 years of age [59]. It enrolled about 20,000 patients since year 2000, mostly patients with T1DM. Treatment Options for T2DM in Adolescents and Youth (TODAY) study assessed comparative efficacy of metformin alone, or in combination with rosiglitazone or lifestyle intervention [60]. A total of 699 participants were enrolled from 2004 to 2009. Both studies found high prevalence of microalbuminuria at initial assessment and progression during follow-up. In the TODAY study, the prevalence of microalbuminuria almost tripled (from 6.3% to 16.6%) over 4 years of follow-up [61]. The SEARCH study showed that in newly diagnosed patients with T2DM, more than 15% of participants had evidence of albuminuria progression during 7 years of follow-up [62]. Albuminuria progression was significantly more aggressive in patients with T2DM than in T1DM. Another SEARCH analysis showed that the odds of having DKD at 7 years after initial diagnosis of diabetes were 2.5 times higher in T2DM than in T1DM [63]. Nephropathy secondary to T1DM is discussed in detail in Chap. XXX.

Long-term kidney complications including incidence of ESKD in patients with childhood onset T2DM were examined in a cohort of Pima Indians [64]. The incidence of diabetic ESKD was 25 cases per 1000 person-years in youth onset diabetes less than 20 years of age and 5.4 cases per 1000 person-years in older onset [64]. ESKD was the second most common (after cardiovascular causes) cause of death in these patients. In another study, long-term kidney outcomes were compared in youth-onset T1DM and T2DM [65]. In this Canadian study, at 25 years follow-up, almost half of the patients with T2DM developed DKD while there were no kidney complications in patients with T1DM. Survival analysis showed that patients with T2DM had four times higher risk of developing any kidney complications as compared to patients with T1DM. The risk to develop ESRD in T2DM patients was 39 times higher when compared to healthy controls. The above studies strongly indicate that T2DM in children and adolescents is associated with a much higher risk of kidney complications than T1DM, and if they have preexisting CKD, this will greatly accelerate CKD progression.

The Effect of Nontraditional Cardiometabolic Factors on Kidney Outcomes

Nonalcoholic Fatty Liver Disease (NAFLD)

Nonalcoholic fatty liver disease (NAFLD) characterized by the accumulation of triglycerides within hepatocytes is the most common cause of pediatric liver disease. In children and adolescents, NAFLD has been consistently associated with obesity and insulin resistance and is a common comorbidity of MS. Children with MS have five times higher odds of having NAFLD than overweight and obese children without MS [66]. NAFLD is associated with all traditional cardiometabolic risk factors including diabetes, impaired fasting glucose, low HDL, high triglycerides, and hypertension [67].

NAFLD may share common pathogenic mechanisms with obesity-induced kidney damage, including lipotoxicity, oxidative stress, pro-inflammatory cytokines, and activation of RAAS. In adults, meta-analysis shows severity-dependent associations of NAFLD with incidence and progression of CKD [68]. Furthermore, NAFLD presents close associations with renal and cardiovascular outcomes in adult patients with pre-existing CKD [69]. In children and adolescents with NAFLD diagnosed by MRI imaging, obese children with NAFLD presented with significantly higher prevalence of estimated GFR <90 ml/min/1.73m^2 (17.5%) compared to obese children without NAFLD (6.7%) or healthy controls (0.77%). Similarly, albuminuria defined as urinary albumin greater than 30 mg/24 h was more frequent in obese children with NAFLD (9.3%) vs. obese children without NAFLD (4%) vs. controls (0%). In multivariable regression analysis, NAFLD was associated with 2.5 higher odds of decreased GFR and/or microalbuminuria independent of adiposity or other MS components [21].

Uric Acid

Elevated uric acid is a common finding in individuals with obesity, hypertension, and MS [70]. Hyperuricemia is found in CKD; however, epidemiological evidence of its role as a cause of CKD progression is inconclusive [70]. The potential mechanisms that uric acid may cause renal injury and hypertension include RAAS activation, inhibition of nitric oxide (NO) synthesis, vascular smooth muscle cell proliferation, and expression of cyclooxygenase-2 in the vascular wall [71]. The above mechanistic pathways promote interstitial fibrosis, afferent arteriopathy, glomerular hypertension, and glomerulosclerosis. In obesity, uric acid may reduce NO bioavailability in adipose cells promoting oxidative stress and insulin resistance [71]. In the National Health and Nutrition Examination Survey (NHANES) adolescent population, hypertension was more prevalent in those with uric acid levels >5.5 mg/dl [72]. Pediatric studies consistently show positive correlation of uric acid levels with BMI and systolic BP [32, 73]. In one randomized clinical trial, urate-lowering therapy in obese adolescents led to reduction of uric acid levels and decrease in BP levels [74]. Hyperuricemia is frequent in children with CKD and associated with increased BMI, hypertension, and albuminuria [75]. In the same study, high uric acid levels were associated with a 4.6 increased odds of having CKD stage 3–5 [75]. In a large study of obese children and adolescents in Italy (CARITALY study), uric acid levels >75th percentile for age and sex were associated with eGFR <90 ml/min/1.73 m^2, presence of NAFLD, or both [76]. Although the causative role of uric acid in the evolution of CKD in MS cannot be established by current evidence, high uric acid levels may be a marker of increased risk for CKD progression in children with MS.

Early Biomarkers of Kidney Injury

MS-related kidney injury is usually asymptomatic in children and adolescents. Recognition of early stages of kidney damage and detection of obese children at risk of adverse future renal outcomes are challenging. Common kidney biomarkers (e.g., serum creatinine or urinary ACR) might not reflect already present kidney involvement. Several candidate serum and urinary biomarkers have been investigated for their predictive value to diagnose kidney damage, including cystatin C, kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), osteopontin, and metalloproteases with inconsistent results [77]. In adolescents with severe obesity without microalbuminuria and normal kidney function, urinary NGAL, IL-18, and KIM-1 were increased prior to bariatric surgery and remained increased 1 year after surgery, suggesting ongoing inflammation and possibly progressing tubulointerstitial fibrosis, despite weight loss and improvement of metabolic profile [78]. Sphingolipid urinary levels suggestive of lipotoxicity-induced glomerular injury were also reported elevated at 1 year after bariatric surgery in adolescents [79]. Still, more evidence is needed to establish the clinical significance of these biomarkers in "silent" kidney injury and their use in routine clinical practice.

Evaluation of Children with MS for Kidney Outcomes

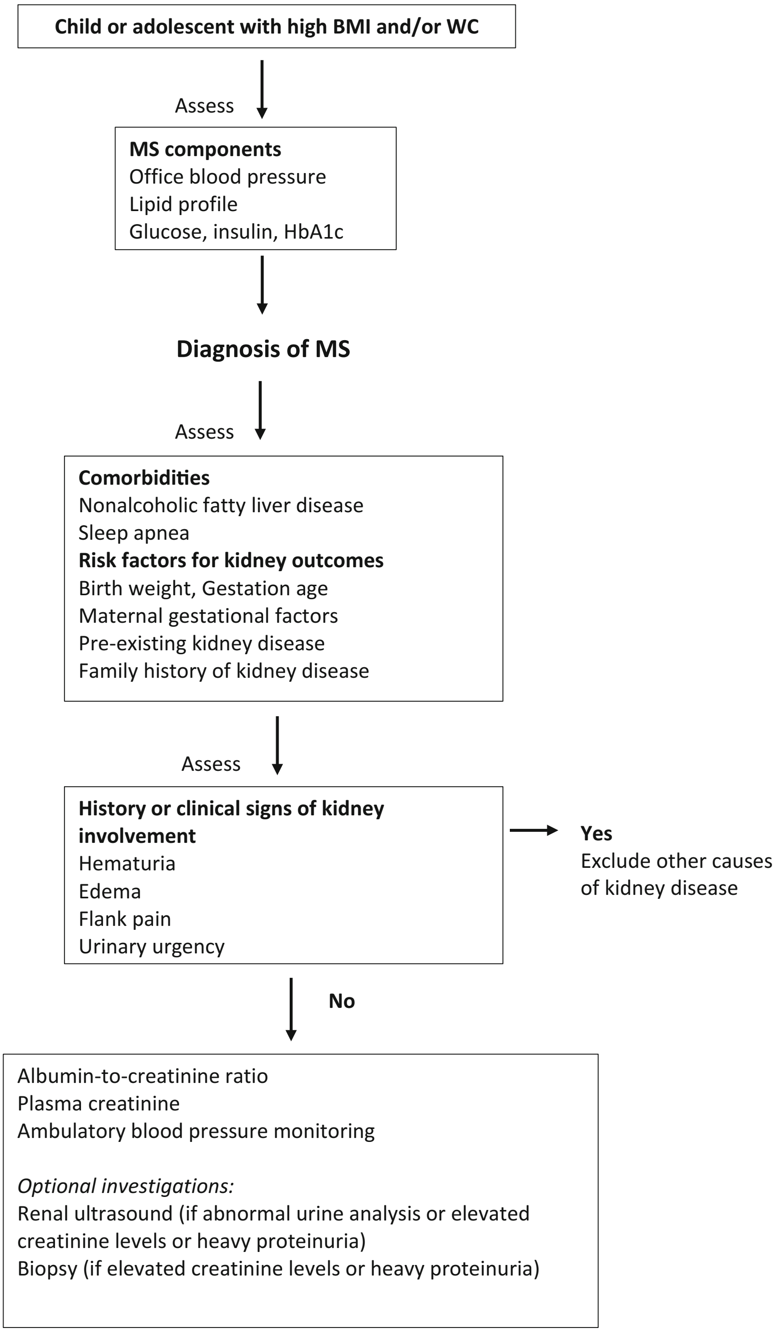
Children and adolescents with MS should undergo evaluation for subclinical kidney damage or evident kidney disease. Frequency of assessment should depend on the number and severity of comorbidities. The presence of morbid obesity, diabetes, hypertension, history of low birth weight and/or prematurity, associated comorbidities (e.g., hyperuricemia), and preexisting kidney disease constitutes a high-risk profile for adverse future kidney outcomes. As described previously, the course of kidney disease in MS is usually asymptomatic for many years. Isolated microalbuminuria and hyperfiltration may be the first and only signs of kidney abnormalities before progression to CKD. Overt or nephrotic range proteinuria is rare in children with MS- and obesity-related kidney injury [6]. If significant proteinuria alone or in combination with hematuria or decreased GFR is present, other disease pathologies besides MS-induced causes of kidney disease should be ruled out (Fig. 2).

Physical examination includes anthropometry, weight, height, BMI calculation, and growth chart evaluation. Blood pressure measurement with an appropriate cuff may be challenging in obese adolescents, especially those with morbid obesity and conical shape arms. As cylindrical cuffs may overestimate BP, tronco-conical cuffs have been proposed for obese individuals with conical shared arms [80]. Acanthosis nigricans may be commonly present, while edema is a rare finding that could be associated with another kidney diagnoses. Dysmorphic features, especially in children with early onset of obesity would guide further evaluation for syndromic obesity such as Bardet-Biedl and Alstrom syndrome that are associated with obesity.

In addition to assessing fasting lipids, serum glucose levels, and HgA1C as part of MS, transaminases and uric acid levels may be included in the initial laboratory assessment to identify children and adolescents with comorbidities that could affect the kidney. Targeted screening for microalbuminuria might be included in the routine assessment of children with MS [6], especially in those with severe obesity and diabetes [23, 73], hypertension [81], or high triglycerides [32]. Assessment of serum creatinine and calculation of GFR would provide information for hyperfiltration state at early stages of kidney involvement, especially in those with diabetes and insulin resistance [23, 76]. If T2DM is among the presenting features of MS, albuminuria and kidney function should be monitored annually [82]. Evaluation with ABPM to detect abnormal BP phenotypes (e.g., white-coat or masked hypertension, nondipping profile, and nocturnal hypertension) should be considered in routine care, especially if sleep apnea, diabetes, or proteinuria are present [83]. Further evaluation including renal imaging and biopsy would be guided by the presence of proteinuria or abnormal kidney function. Finally, echocardiographic evaluation for left ventricular hypertrophy could be considered to complete the assessment of cardiovascular involvement with MS.

Treatment of MS-Induced Kidney Injury

The treatment of MS in children and adolescents, including those with CKD, should target each cardiometabolic component (Table 2). Lifestyle modification with diet, increased physical activity, and behavioral therapy remains the cornerstone of management. Weight loss ideally targets a BMI below the 85th percentile. Even a small initial decrease in BMI has been proven beneficial if maintained and could result in a decrease in cardiometabolic risk factors. A BMI-SDS reduction of 0.25 or greater significantly improved



Child or adolescent with high BMI and/or WC
Assess
MS components
Office blood pressure
Lipid profile
Glucose, insulin, HbA1c
Diagnosis of MS
Assess
Comorbidities
Nonalcoholic fatty liver disease
Sleep apnea
Risk factors for kidney outcomes
Birth weight, Gestation age
Maternal gestational factors
Pre-existing kidney disease
Family history of kidney disease
Assess
History or clinical signs of kidney involvement
Hematuria
Edema
Flank pain
Urinary urgency
Yes
Exclude other causes of kidney disease
No
Albumin-to-creatinine ratio
Plasma creatinine
Ambulatory blood pressure monitoring
Optional investigations:
Renal ultrasound (if abnormal urine analysis or elevated creatinine levels or heavy proteinuria)
Biopsy (if elevated creatinine levels or heavy proteinuria)

Fig. 2 Evaluation algorithm of children and adolescents for MS-related kidney disease

hypertension, hypertriglyceridemia, and low HDL-cholesterol, whereas an increase of BMI-SDS greater than 0.5 doubled the effect in 1388 overweight children who underwent in 1 year lifestyle modification in a specialized obesity clinic [84].

Salt reduction according to the age-recommended daily dietary intake by WHO should be considered in children with MS [85]. Obese children usually consume high amounts of salt [86], and salt reduction may have beneficial effect on both weight and BP, especially in children with

Table 2 Treatment of metabolic syndrome components [9, 90, 91]

MS component	Obesity	Dyslipidemia (High triglycerides/ Low HDL)	Hypertension	Impaired glucose metabolism / type 2 diabetes mellitus
Lifestyle modification	**Gradual weight loss or maintenance**	**Weight management to achieve BMI < 85th pc**	**Weight management to achieve BMI < 85th pc**	**Weight management to achieve BMI < 85th pc**
	Diet (Adequate calories for growth; Total fat 25% to 35% of calories, saturated fat <7% of calories, transfat <1% of calories, cholesterol >300 mg/day; Low salt and sugar intake, high vegetable and fiber intake) **and physical activity (**1 h of moderate-to-vigorous each day, limit screen time limited to <2 h/ day)	**Diet** (Decreased simple sugars and low saturated and transfats diet) **and physical activity**	**Diet** (Low salt intake, increased consumption of fruits and vegetables) **and physical activity**	**Diet and physical activity**
Pharmacological treatment	Orlistat >12 years of age	Fibrate, fish oil if triglycerides >500 mg/dl or > 400 mg/dl in adolescents with type 2 diabetes mellitus	ACEi, ARB, CCB, and thiazide-like diuretics	Metformin if repeated fasting glucose 100–126 mg/dl, Insulin
2nd line therapies	Bariatric surgery		Mineralocorticoid receptor blockers (spironolactone, eplerenone)	

Abbreviations: BMI body mass index, TG triglycerides, HDL high density lipoprotein, ACE angiotensin converting enzyme inhibitors, ARB angiotensin receptor blocker, CCB calcium channel blocker, FG fasting glucose

CKD. A Cochrane review has shown that salt restriction results in decrease of BP and urinary protein excretion in patients with existing CKD [87]. Dietary Approaches to Stop Hypertension (DASH), which emphasize reduction of sodium intake along with increased fruits, vegetables, and low-fat milk products consumption, have beneficial effect on weight reduction and BP levels in adolescent cohort studies [88] and have been associated with a lower incidence of CKD in the general adult population [88]. On the other hand, high protein diets for weight loss should be avoided in children and adolescents with MS as this enhances hyperfiltration and is associated with rapid kidney decline in the general population [89].

In clinical practice, a significant proportion of children and adolescents may fail to comply with lifestyle medication or may regain weight after the end of lifestyle intervention program. Several reasons have been proposed including lack of family involvement, psychological issues, and even physiological hormonal adaptation during weight loss [90].

Pharmacological treatment for cardiometabolic risk factors should be considered in children and adolescents with MS, especially if kidney damage is present. Angiotensin Converting Enzyme (ACE) inhibitors or Angiotensin II receptor Blockers (ARB)s are considered the first-line treatment in the case of obesity-induced hypertension and may also have a beneficial reno-protective effect in youth with MS [81, 91]. Inhibition of RAAS may improve blood flow to muscles, decrease sympathetic nervous system activity, and improve insulin sensitivity. These agents may also have PPAR-γ effect on insulin sensitivity and are associated with a lower risk of diabetes [91]. Lipid treatment targeting low-density lipoprotein (LDL) cholesterol is also suggested for children and adolescents with MS [9]. Kidney Disease Improving Global Outcomes (KDIGO) guidelines suggest using statins in children with CKD aged $\geq$10 years with severely elevated LDL cholesterol, especially in those with multiple additional risk factors such as a family history of premature coronary disease, diabetes, hypertension, smoking, and ESKD [92]. Pharmacological treatment for high triglycerides levels, the main feature of dyslipidemia in children and adolescents with MS, is recommended in those with levels above 400 mg/dl [9, 82]. In adolescents with MS and T2DM, metformin is recommended in addition to lifestyle modification in those with an eGFR >30 ml/min/1.73m^2 [82]. New promising nephroprotective agents such as glucagon-like peptide 1 analogues and sodium-glucose cotransporter 2 inhibitors have shown significant reduction in cardiovascular and renal-specific outcomes in clinical trials of adults with T2DM [93]. These agents have not yet been evaluated in children and adolescents. Modifying hyperuricemia may have beneficial effect on BP levels in children with MS [74], but solid evidence on the protective effect against adverse kidney outcomes is lacking.

While treatment of hypertension, dyslipidemia, and abnormal glucose metabolism in children with MS is important, weight loss should be the main goal of treatment, including those with preexisting kidney abnormalities. It results in a reduction of proteinuria and microalbuminuria in obese individuals. Most of the data of the effect of conservative treatment on weight loss, diet, and physical activity interventions on kidney outcomes are reported in adults. Metaregression analysis in adults showed that 1 kg weight loss was independently associated with reduction by 110 mg in proteinuria and by 1.1 mg in microalbuminuria, respectively, but had no effect on creatinine clearance [94].

In youth, most of the data related to the effect of weight reduction on kidney outcome comes from studies in adolescents who underwent bariatric surgery. Bariatric surgery in adolescents can prevent or improve many health problems, including kidney abnormalities [95]. The results from the Teen Longitudinal Assessment of Bariatric Surgery (Teen-LABS) study showed improvement in albuminuria and kidney function. Prior to surgery, 14% of the cohort had microalbuminuria, 3% had macroalbuminuria, 3% had eGFR$<$60 ml/min/1.73 m^2 and 7.1% had eGFR$>$150 ml/min/1.73 m^2 [78]. At 3 years follow-up postsurgery, in participants with a preoperative eGFR $<$90 ml/min/1.73m^2, eGFR improved from a mean of 76–102 ml/min/1.73m^2 [96]. In those with elevated albuminuria at baseline (ACR $\geq$30 mg/g), the median ACR decreased from 74 to 17 mg/g. In another study, 5-year kidney outcomes were compared in adolescents with severe obesity and T2DM enrolled in the Teen-LABS (bariatric surgery) and TODAY (medical management) studies [97]. At baseline, elevated albuminuria was present in 21% and increased to 43% at 5 years in TODAY participants. In contrast, albuminuria decreased from 27% of Teen-LABS diabetic participants prior to surgery to 5% at 5 years follow-up. At 5 years, there were 27-fold higher odds of DKD in TODAY participants compared to Teen-LABS participants. A recent analysis of bariatric surgery in adults (LABS study) and adolescents (Teen-LABS) showed that adolescents experienced earlier remission of elevated albuminuria than adults [98]. These data indicate that bariatric surgery improves kidney outcomes in youth with severe obesity, including those with MS and T2DM, and may support the recommendation for bariatric surgery in adolescents who are near final-adult height and attained pubertal development if they have

$BMI \geq 40\ kg/m^2$ or $BMI \geq 35\ kg/m^2$ with significant obesity-related comorbidities who failed to respond to dietary or behavior modifications [95].

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Renal Manifestations of Metabolic Disorders in Children

31

Francesco Emma, Matthias R. Baumgartner, Diego Martinelli, and Carlo Dionisi-Vici

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Abstract

While the majority of children with renal dysfunction have structural, immunological, or infectious disorders, some have metabolic defects arising from abnormalities in biochemical pathways of cell metabolism. This chapter focuses on metabolic diseases that compromise kidney function or cause renal structural abnormality and are not covered elsewhere in the textbook.

Keywords

Metabolism · Inborn errors of metabolism · Enzyme · Metabolic pathway

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Introduction

While the majority of children with renal dysfunction have structural, immunological, or infectious diseases, a minority of patients have metabolic defects arising from abnormalities in biochemical pathways of cell metabolism. Progress in the treatment of many of these patients has considerably improved their survival and has often revealed renal symptoms that were not included in the original descriptions of the diseases. Pediatric nephrologists should remain vigilant to the possibility of a metabolic disease, especially when extra-renal symptoms are present. Kidney involvement in metabolic diseases is very heterogeneous. Renal tubules, in particular proximal tubules, have very high metabolic rates; hence, they are very sensitive to diseases that compromise cell energy production. Mutations in solute carriers that control metabolic pathways in other organs may cause tubulopathies. In other cases, metabolic dysfunctions cause nephrocalcinosis or urolithiasis. Glomerular lesions can result from abnormal depositions of metabolites, defects in the synthesis of structural components, or toxic damage to podocytes and endothelial cells. More rarely, metabolic diseases interfere in utero with normal embryogenesis, causing renal malformations. In many diseases, kidney involvement remains subclinical, even if substrate accumulation or enzyme defects can be demonstrated directly in the renal parenchyma. Sometimes, changes in urine color or odor are the only symptom. Finally, renal involvement can be secondary to damage to other organs, as in metabolic diseases that cause acute rhabdomyolysis or hemolysis.

Owing to the complexity of metabolic pathways, a large number of defects have been characterized at the molecular level. This has created the need to develop classifications that have been periodically updated. The latest revision includes 1015 well-characterized inborn errors of metabolism and 111 less well-characterized conditions [1]. Diseases are grouped along major metabolic pathways, since defects belonging to the same pathway are likely to share clinical features and may respond to similar treatments.

This chapter concentrates on diseases that are associated with renal and urological abnormalities and are not covered elsewhere in the textbook (i.e., cystinosis, Fabry disease, primary hyperoxaluria, galactosemia, tyrosinemia type I, hereditary fructose intolerance, glycogen storage disease type I, Fanconi–Bickel syndrome, methylmalonic acidemia, Wilson disease, amino acidurias, and mitochondrial cytopathies). Diseases are illustrated following the latest nosology for inborn errors of metabolism [1]. Given their elevated number, most diseases are only listed in Table 1, along with their most relevant renal and extra-renal symptoms; the OMIM reference ID is provided to facilitate retrieval of additional information. A selected number of diseases are also illustrated in more detail in the text below.

Congenital Diseases of Glycosylation

Congenital diseases of glycosylation (CDG) are a group of inherited multisystem diseases due to defective glycoprotein and glycolipid glycan assembly and attachment [2–4]. Protein glycosylation includes the addition of N-linked glycans, O-linked glycans, phosphorylated glycans, glycosaminoglycans, and glycosylphosphatidylinositol anchors to peptide backbones; glycolipids are obtained through the addition of sugars to lipids [5]. Different CDG subtypes have been described (see Table 1). The clinical presentation can be very heterogeneous, including cases presenting with cerebellar atrophy, epilepsy, movement diseases, multiple exostoses, progeria, developmental delay, or fatal multisystem diseases [4]. The most common CDG is a N-linked glycosylation disease termed PMM2-CDG (also known as CDG1a). Patients may present with dimorphisms, hypotonia, failure to thrive, diarrhea, abnormal fat pads, inverted nipples, abnormal eye movements, hepatomegaly, and cardiomyopathy. Laboratory investigations often show hypothyroidism and olivo-ponto-cerebellar atrophy [3, 4, 6]. The isoelectric focusing of serum transferrin is the first screening test to diagnose most CDGs, although it does not identify defects of O-linked glycosylation and other extremely rare forms. If abnormal, specific

Table 1 Metabolic diseases with renal involvement

Disease	OMIM	Gene	Inher.	Main features	Renal features	Tub.	Glom.	Int.	UL/ NC	CAKUT
Disorders of nitrogen-containing compounds										
Cystinosis (nephropathic)	219800	CTNS	AR	Failure to thrive, vomiting, rickets, often blond – fair hair, corneal cystine crystals, multisystem involvement	Fanconi syndrome, CDK	+	+	+		
Cystinosis (juvenile)	219900	CTNS	AR	Corneal crystals, retinopathy	Glomerulopathy, mild proximal tubular dysfunction	+	+			
Tyrosinaemia type 1	276700	FAH	AR	Hepatomegaly, acute/ chronic liver disease, hepatocellular carcinoma, rickets, neurological crises	Fanconi syndrome, CDK	+				
Lysinuric protein intolerance	222700	SLC7A7	AR	Failure to thrive, hepatosplenomegaly, vomiting, refusal of protein-rich foods, interstitial pneumopathy/ alveolar proteinosis, immune dysfunction, encephalopathy	Tubulopathy, CDK, hypertension, immune mediated glomerulonephritis, tubulo-interstitial nephritis.	+	+	+		
Cystinuria	220100	SLC3A1 SLC7A9	AR		Nephrolithiasis				+	
Classical homocystinuria	236200	CBS	AR	Marfan-like phenotype, lens dislocation, skeletal abnormalities, livedo reticularis, seizures, mental retardation, psychiatric disorders, cerebrovascular accident, large vessel thrombosis	Renal vein thrombosis					

(continued)

Table 1 (continued)

Disease	OMIM	Gene	Inher.	Main features	Renal features	Tub.	Glom.	Int.	UL/ NC	CAKUT
Propionic acidemia	606054	PCCA PCCB	AR	Vomiting, hypotonia, failure to thrive, hyperammonemic coma, encephalopathy, cardiomyopathy	CDK (adult)			+		
Methylmalonic acidemia	251000 251100 251110	MUT MMAA MMAB	AR	Vomiting, hypotonia, failure to thrive, hyperammonemic coma, encephalopathy, cardiomyopathy	Tubulopathy, tubulo-interstitial changes, CDK	+		+		
Prolidase deficiency	170100	PEPD	AR	Rash, dysmorphism, anemia, splenomegaly, recurrent infections, systemic lupus erythematosus	Lupus nephritis		+			
Alkaptonuria	203500	HGD	AR	Ochronotic pigmentation of cartilage and collagen, arthritis	Urine turns black if alkaline, Nephrolithiasis in adults				+	
Homocystinuria	236200	CBS	AR	Ectopia lentis, skeletal abnormalities, Marfan-like features, neuropsychiatric symptoms, thromboembolism	Renal infarction, hypertension					
Glutathione synthetase deficiency	266130	GSS	AR	Mental retardation, ataxia, retinopathy	Nephrolithiasis				+	
Adenosine deaminase deficiency	102700	ADA	AR	Severe combined immunodeficiency	Transient renal tubular acidosis, proteinuria, mesangial sclerosis		+			
Lesch Nyhan syndrome hypoxanthine-guanine phosphoribosyl transferase (HGPRT) deficiency	300322	HPRT1	X-L	Lesch Nyhan syndrome (complete deficiency): developmental delay, choreo-athetoid movements, self-mutilation	Nephrolithiasis, CDK				+	

				Partial deficiency: gouty arthritis						
Adenine phosphoribosyl-transferase deficiency	614723	APRT	AR		Nephrolithiasis (2,8-dihydroxyadenine) AKI, CKD				+	
Hereditary orotic aciduria	258900	UMPS	AR	Megaloblastic anemia, immunodeficiency, failure to thrive	Obstructive uropathy, orotic acid crystalluria, hematuria, urolithiasis				+	
Xanthinuria type 1	278300	XDH	AR	Myopathy, arthropathy	Nephrolithiasis, AKI				+	
Xanthinuria type 2	603592	XDH-AO	AR	Myopathy (rare)	Nephrolithiasis, AKI				+	
Phosphoribosylpyrophosphate synthetase superactivity	300661	PRPS1	X-L	Neurological deterioration, deafness, dysmorphic features, gout	Uric acid stones				+	
Urate transporter-1 deficiency	220150	SLC22A12	AR	Exercise-induced acute renal failure with acute tubular necrosis	Tubular defect in presecretory reabsorption of urate, Nephrolithiasis, AKI				+	
Urate voltage-driven efflux trsported 1	612076	SLC2A9	AD, AR	Exercise-induced acute renal failure (in some patients	Nephrolithiasis, AKI				+	
Disorders of vitamins, cofactors, metals, and minerals										
Imerslund-Grasbeck syndrome	261100	CUBN AMN	AR	Malabsorption of vitamin B12, megaloblastic anemia, peripheral neuropathy	Low molecular weight proteinuria	+				
Cobalamin deficiencies cblC, cblD, cblF, cblE, cblG, cblJ	277400 277410 236270 277380 250940 614857	MMACHC MMADHC MTRR LMBRD1 MTR ABCD4	AR	Developmental delay, hydrocephalus, microcephaly, visual impairment, nistagmus, dysmorphic features, megaloblastic anemia, pancytopenia, cardiomyopathy, pulmonary hypertension	HUS, glomerulopathy, tubulopathy	+		+		
MTHDH deficiency	172460	MTHFD1	AR	Megaloblastic anemia, severe combined immune deficiency	HUS		+			

(continued)

Table 1 (continued)

Disease	OMIM	Gene	Inher.	Main features	Renal features	Tub.	Glom.	Int.	UL/ NC	CAKUT
Hypophosphatasia (infantile form)	241500	ALPL	AR	Rickets, developmental delay, hypercalcemia, craniosynostosis	Hypercalciuria, nephrocalcinosis				+	
Wilson's disease	277900	ATP7B	AR	Liver disease, neurological symptoms, Kayser-Fleischer ring, hemolytic anemia, cardiomyopathy	Fanconi syndrome, proteinuria	+				
Menke's disease	309400	ATP7A	X-L	Kinky hair, developmental delay and regression, seizures, skin laxity and hypopigmentation, hernia	Bladder or ureteric diverticuli, nephrolithiasis, urinary infections, CDK			+	+	+
Molybdenum cofactor deficiency	252150	MOCS1	AR	Severe developmental delay microcephaly, seizures, spasticity	Xanthine stones, Increased urinary xanthine, hypoxanthine, S-sulfocysteine				+	
Glutaric acidemia type 2	231680	ETFDH ETFA ETFB	AR	Neonatal acidosis, hypoglycemia, liver dysfunction, hypotonia, dysmorphic features	Renal cystic disease			+		
Disorders of carbohydrates										
Galactosemia	230400	GALT	AR	Liver failure, cataract, failure to thrive, neonatal E. Coli sepsis, speech delay, ovarian insufficiency	Renal proximal tubular dysfunction	+				
Hereditary fructose intolerance	229600	ALDOB	AR	Liver failure, aversion for fructose-containing foods, post-prandial vomiting and hypoglycemia	Renal proximal tubular dysfunction	+				

Fanconi renotubular syndrome 4 with maturity-onset diabetes of the young	616026	HNF4A	AD	Neonatal hyperinsulinism/ hypoglycemia, diabetes mellitus (some patients), macrosomia	Renal proximal tubular dysfunction nephrocalcinosis	+				
Renal cysts and diabetes syndrome	137920	HNF1B	AD	Early-onset diabetes mellitus, pancreatic dysfunction, female genital tract malformations, abnormal liver function tests (subclinical), hyperuricemia and gout, abnormal behavior (if 17q12 deletion)	Renal and urinary tract malformations, renal cysts, hypomagnesemia/ hypermagnesuria	+				+
Glycogen storage disease type 1A	232200	G6PG	AR	Hypoglycemia, hepatomegaly, hypercholesterolemia, hypertriglyceridemia, hyperuricemia, lactic acidosis	Nephromegaly, gout nephropathy, renal stones, nephrocalcinosis, Fanconi syndrome, glomerular hyperfiltration, CKD	+	+		+	
Glycogen storage disease type 1B	232220	SLC37A4	AR	Hypoglycemia, hepatomegaly, hypercholesterolemia, hypertriglyceridemia, hyperuricemia, lactic acidosis, neutropenia, inflammatory bowel disease, recurrent infections	Nephromegaly, glomerular hyperfiltration, nephrolithiasis, CKD		+		+	
Fanconi-Bickel	227810	SLC2A2	AR	Severe growth retardation, liver disease, hypoglycemia, rickets	Fanconi syndrome	+				

(continued)

Table 1 (continued)

Disease	OMIM	Gene	Inher.	Main features	Renal features	Tub.	Glom.	Int.	UL/ NC	CAKUT
Mitochondrial disorders of energy metabolism (see also chapter…)										
GRACILE syndrome	603358	BCS1L	AR	Severe growth retardation, cholestasis, liver hemosiderosis	Tubulopathy	+				
Pyruvate carboxylase deficiency (some forms)	266150	PC	AR	Metabolic acidosis, neurological symptoms, hepatomegaly	Tubulopathy, CKD	+		+		
Kearns Sayre syndrome	530000	Large mtDNA deletion	Spor.	Ophthalmoplegia, retinal degeneration, heart block, ataxia, hypoparathyroidism	Bartter-like phenotype, hypomagnesemia	+				
MELAS	540000	m.3243A > G m.3271 T > C	MI	Stroke-like episodes, encephalopathy, deafness, macular dystrophy, myopathy, diabetes mellitus	FSGS		+			
MIDD	520000	m.3243A > G m.14709 T > C m.8396A > G	MI	Maternal inherited diabetes mellitus, deafness	FSGS		+			
Neonatal mitochondrial encephalopathy, cardiomyopathy	614052	TMEM70	AR	Hypospadia, criptorchidism, dysmorphic features, cardiomyopathy, pulmonary hypertension	CKD					+
Mitochondrial complex IV deficiency	220110	Multiple genes	AR, MI	Deafness, blindness, cardiomyopathy, liver dysfunction, hypotonia, developmental delay, encephalopathy, Leigh syndrome, epilepsy, myopathy, failure to thrive	Tubulopathy	+				
HUPRA syndrome	613845	SARS2	AR	Developmental delay, gastrointestinal	Hyperuricemia, alkalosis, CKD					

				dysmotility, pulmonary hypertension						
Coenzyme Q10 deficiency-CoQ2	607426	COQ2	AR	Encephalomyopathy, liver failure, deafness	SRNS		+			
Coenzyme Q10 deficiency-CoQ6	614650	COQ6	AR	Deafness, seizures	SRNS		+			
Coenzyme Q10 deficiency-CoQ9	614654	COQ9	AR	Encephalomyopathy, cardiomyopathy	Tubulopathy	+				
Coenzyme Q10 deficiency-PDSS1	615573	ADCK4/ COQ8B	AR		SRNS		+			
Coenzyme Q10 deficiency-PDSS2	614652	PDSS2	AR	Leigh syndrome, blindness	SRNS		+			
Mitochondrial ribonucleotide sub.2	612075	RRM2B	AR	Dysmyelination, seizures, failure to thrive, ophtalmoplegia, ptosis, myopathy	Tubulopathy	+				
Pearson syndrome	557000	Large mtDNA deletion	Spor.	Pancytopenia, pancreatic dysfunction	Proximal tubulopathy	+				
Complex III deficiency	124000	BCS1L	AR	Encephalopathy, liver dysfunction, failure to thrive	Tubulopathy	+				
Complex III deficiency	615824	UQCC2	AR	Dysmorphic features, deafness, developmental delay	Proximal renal tubular acidosis	+				
Mitochondrial ribosomal protein deficiencies	610498 611719 617664 615395	MRPS16 MRPS22 MRPS34 MRPL44	AR	Developmental delay, cardiomyopathy, hypotonia, oedema, ascitis	Tubulopathy	+				
mtDNA depletion	603921	SUCLA2	AR	Leigh syndrome, deafness, dystonia	Tubulopathy	+				
mtDNA depletion	251880	DGUOK	AR	Liver dysfuncion, abnormal eye movements, developmental delay	Tubulopathy	+				
mtDNA depletion	256810	MPV17	AR	Hypoglycemia, liver dysfunction, developmental delay	Tubulopathy	+				

(continued)

Table 1 (continued)

Disease	OMIM	Gene	Inher.	Main features	Renal features	Tub.	Glom.	Int.	UL/ NC	CAKUT
mtDNA depletion	612075	RRM2B	AR	Encephalopathy, seizures, impaired vision, failure to thrive	Proximal tubulopathy	+				
Disorders of lipids										
Carnitine palmitoyl transferase deficiency 2	608836	CPT2	AR	Microcephaly, cataract, cardiomyopathy, liver dysfunction, myopathy	Enlarged polycystic kidneys, renal dysplasia, hydronephrosis, lipid accumulation in kidneys, CKD	+		+		+
Long-chain fatty acid oxidation disorders	201475 609016 609015	ACADVL HADHA HADHB	AR	Liver disease, hypoketotic hypoglycemia, cardiomyopathy, myopathy, encephalopathy, retinopathy, neuropathy, hypoparathyroidism, maternal HELLP syndrome	Renal tubulopathy secondary to myoglobinuria, AKI	+				
Acure recurrent myoglobinuria	268200	LPIN1	AR	Fever-induced myoglobinuria	Recurrent AKI	+				
MECRCN	616878	TANGO2	AR	Dysmorphic features, deafness, cardiac arrhythmias, cardiomyopathy, fever-induced acute rhabdomyolysis, developmental delay, myopathy, pyramidal signs	Recurrent AKI	+				
Smith-Lemli-Opitz syndrome	270400	DHCR7	AR	Facial dysmorphism, hypotonia, cataract, mental retardation, abnormalities of limbs, brain, genitalia	Cystic dysplasia, hypoplasia, agenesis, duplication, PUJ obstruction, VUR					+

Conradi Hunermann syndrome	302960	EBP	X-L	Dysmorphic features, skeletal dysplasia, cataract, ichthyosis, mental retardation	Renal hypodysplasia, hydronephrosis					+
CHILD syndrome	308050	NSDHL	X-L	Hemihypertrophy, ichythyosiform erythroderma, limb defects	Renal hypodysplasia					+
Desmosterolosis	602398	DHCR24	AR	Dysmorphic features, developmental delay, skeletal dysplasia, arthrogryposis, osteosclerosis	Renal hypodysplasia, Ambigous genitalia					+
Latosterolosis	607330	SC5DL	AR	Dysmorphic features, cataract, chronic liver dysfunction, hexadactily, mental retardation	Hypospadia					+
Antley-Bixler syndrome with genital anomalies	201750	POR	AR	Dysmorphic features, skeletal dysplasia	Hydronephrosis, renal hypodysplasia					+
Lecithin:Cholesterol acyltransferase deficiency	245900	LCAT	AR	Corneal lipid deposits, corneal opacities	Proteinuria, hematuria, renal failure		+			
Lipoprotein glomerulopathy	611771	APOE		Elevated apolipoprotein E, usually in patients of Asian ancestry	Nephrotic syndrome (resistant to treatment), hematuria, hypertension, renal failure, glomerular lipoprotein thrombi		+			
Enoyl-coenzyme A hydratase/ L-3-hydroxyacyl-coenzyme A dehydrogenase (EHHADH) deficiency	615605	EHHADH	AD	Short stature	AD Fanconi syndrome (mutations causing mistargeting to mitochondria)	+				
Disorders of tetrapyrroles										
Doss hepatic porphyria	612740	ALAD	AR	Abdominal pain, constipation, vomiting, neurologic features, muscle pain	Urine may turn red/purple Hypertension					

(continued)

Table 1 (continued)

Disease	OMIM	Gene	Inher.	Main features	Renal features	Tub.	Glom.	Int.	UL/ NC	CAKUT
Acute intermittent porphyria	176000	HMBS	AD	Abdominal pain, constipation, recurrent acute psychiatric manifestations, neuropathy	Urine may turn red/purple AKI, CKD, urinary retention, hypertension					
Storage disorders										
Fabry's disease	301500	GLA	X-L	Acroparesthesias, angiokeratoma, hypohidrosis, stroke-like episodes, corneal verticillate, abdominal pain, angina, cardiomyopathy, cramps	Tubulopathy, glomerulopathy, CKD, parapelvic cysts	+	+			
Infantile sialic acid storage disease	269920	SLC17A5	AR	Hydrops, edema, dysmorphic features, hepatosplenomegaly, hypotonia, developmental delay, failure-to-thrive, skeletal dysplasia	Proteinuria, nephrotic syndrome		+			
Action myoclonus renal failure syndrome	254900	SCARB2	AR	Myoclonus, tremor, ataxia, horizontal saccades, dysphagia, no cognitive deterioration	Proteinuria, nephritic syndrome, FSGS		+			
Vici syndrome	242840	EPG5	AR	Corpus calloses agenesis, cataract, developmental delay, failure to thrive, hypopigmentation, immune deficiency, myopathy, cardiomyopathy, lip/palate cleft	Renal tubular dysfunction	+				
Disorders of peroxisomes and oxalate										
Peroxisome biogenesis disorders – Zellweger's	Multiple	PEX genes (multiple)	AR	Facial dysmorphism, hypotonia, severe	Renal cysts, CKD			+		

spectrum disorders (including NALD, IRD)				developmental delay, brain dysplasia, seizures, cataract, retinopathy, deafness, cholestatic liver disease, adrenal insufficiency						
Primary Hyperoxaluria types 1,2,3	259900 260000 613616	AGXT GRHPR HOGA1	AR	Optic nerve atrophy, retinopathy, heart block, pathologic fractures, peripheral neuropathy	Nephrolithiasis, nephrocalcinosis, CKD			+	+	
Disorders of glycosylation										
Congenital disorder of glycosylation type 1a	212065	PMM2	AR	Ataxia, olivopontocerebellar hypoplasia, peripheral neuropathy, strabismus, inverted nipples, hepatomegaly, 'Orange peel' skin, abnormal subcutaneous fat tissue distribution, mental retardation, primary ovarian failure, prolonged prothrombin time	Proteinuria (may be congenital), microcysts		+	+		
Congenital disorder of glycosylation type 1 h	608104	ALG8	AR	Intrauterine growth retardation, neonatal, ascites protein-losing enteropathy, cerebellar hypoplasia, hepatomegaly, cataract	Renal cysts, tubulopathy	+		+		
Congenital disorder of glycosylation type 1 l	608776	ALG9	AR	Brain atrophy, epilepsy, mental retardation, hepatosplenomegaly	Renal cysts			+		
Congenital disorder of glycosylation type 1 k	608540	ALG1	AR	Intrauterine growth retardation, non-immune fetal hydrops, large fontanel, microcephaly, micrognathia, dysmorphic features,	Nephrotic syndrome, congenital (in some patients)		+			

(continued)

Table 1 (continued)

Disease	OMIM	Gene	Inher.	Main features	Renal features	Tub.	Glom.	Int.	UL/ NC	CAKUT
				partial blindness, cardiomyopathy, hepatosplenomegaly, hypogonadism, severe psychomotor retardation, seizures, cerebral atrophy						
B3GALTL – Congenital disorder of glycosylation (Peter-plus syndrome)	261540	B3GALTL	AR	Dysmorphic features, developmental delay, short limb dwarfism, cataract, coloboma, skeletal dysplasia	Hydronephrosis, hydrourether, kidney/ ureteral duplication					+
Congenital disorder of glycosylation type 2d	607091	B4GALT1	AR	Psychomotor and mental retardation, macrocephaly due to Dandy-Walker malformation, hypotonia, coagulopathy, myopathy with elevated creatine kinase	Nephrotic syndrome		+			
Congenital disorder of glycosylation type 2e	608779	COG7	AR	Microcephaly, epilepsy, developmental delay, hepatomegaly, failure to thrive, ventricular septal defect	Neurogenic bladder, obstructive uropathy, renal tubulopathy	+				+

Congenital disorder of glycosylation type 2f	603585	SLC35A1	AR	Epilepsy, mental retardation, macrothrombocytopenia	Proteinuria		+			
Congenital disorder of glycosylation type 2 k	614727	TMEM165	AR	Psychomotor and growth retardation,midface hypoplasia, muscle weakness, fat excess, joint laxity, and hepatosplenomegaly with increased serum transaminases, increased creatine kinase and partial growth hormone deficiency, skeletal dysplasia, white matter abnormalities and hypoplasia of the pituitary gland	Nephrotic syndrome		+			
Polycystic kidney disease 3	600666	GANAB	AD	Hypertension, liver cysts, liver dysfunction	Renal cysts, CKD (rare)			+		

Abbreviations
Inher. Inheritance, *Tub*. Tubular disease, *Glom*. Glomerular disease, *Int*. Interstitial disease, *UL/NC* Urolithiasis/Nephrocalcinosis, *CAKUT* Congenital Abnormalities of the Kidneys and Urinary tract, *CKD* Chronic Kidney Disease, *AKI* Acute Kidney Disease, *AR* Autosomal Recessive, *AD* Autosomal Dominant, *X-L* X-linked, *MI* Maternal Inheritance, *Spor*. Sporadic, *FSGS* focal segmental glomerulosclerosis, *SRNS* steroi-resistant nephrotic synrome, *PUJ* pelvic ureteric junction

enzymatic and genetic tests should be performed [2–4].

The most common renal abnormality in CDGs is the presence of microcysts that give a hyperechogenic appearance to kidneys by ultrasonography and is usually observed in children with multisystemic involvement [6–8]. Cysts are located in the cortex, arise predominantly from tubules, and vary from case to case in number and size, even within the same family [7, 8]. Kidneys may be enlarged; occasionally, isolated cysts have been reported [6, 9]. In a large review of published PMM2-CDG cases, 56/933 patients were reported to have renal involvement [9]. Renal abnormalities were congenital in 41/56 cases [9]. Cystic kidney disease and mild proteinuria were the most common findings. Six patients presented with congenital nephrotic syndrome; postmortem biopsy was available in one case, showing diffuse mesangial sclerosis [9]. Proteinuria in PMM2-CDG contributes to the edema and ascites that these patients frequently develop [5]. Nephrotic syndrome has also been reported in B4GALT1-CDG, ALG1-CDG, and TMEM165-CDG [10–12]. Renal cysts have been reported in more rare defects, namely, in ALG6-CDG, ALG8-CDG, ALG9-CDG, and GANAB-CDG [9]. Patients with B3GALTL-CDG may present with hydronephrosis and other urological abnormalities; patients with ALG8-CDG and SLC35A1-CDG may develop isolated proteinuria; neurogenic bladder, obstructive uropathy, and tubulopathies have been reported in patients with COG7-CDG [3]. Transferrin isoelectric focusing should therefore be added to the list of investigations of patients with renal cysts, kidney malformations, isolated proteinuria, or nephrotic syndrome that present with multisystemic diseases or syndromic features.

Diseases of Purine and Pyrimidine Metabolism and Urate Transport

Children who develop uric acid urolithiasis, acute renal failure in the neonatal period, or crystal nephropathy may require investigation of their purine metabolism. Purines are involved in the synthesis of nucleotides and coenzymes, in signal transduction (e.g., cAMP) and in the generation of ATP. The metabolic end-product of these pathways, uric acid, and its immediate precursor, xanthine, are insoluble in urine. Therefore, overproduction predisposes to the development of urinary crystals and stone formation. Uric acid reabsorption in proximal tubules is age- and sex-dependent, with children reabsorbing less of the filtered urate load and consequently having lower plasma urate concentrations, but more uricosuria [13, 14]. As glomerular filtration rate declines, fractional excretion of urate increases [14]. Urolithiasis is the commonest renal manifestation of diseases of purine nucleotide biosynthesis, savage pathway, and breakdown. A rare disease of pyrimidine biosynthesis, UMPS deficiency, may also present with urolithiasis.

Diseases of the Purine Savage Pathway

This group of diseases includes adenine phosphoribosyl transferase (APRT) deficiency [15] and hypoxanthine-guanine phosphoribosyl transferase (HPRT) deficiency [16] (see chapter "Urolithiasis and Nephrocalcinosis"). Children may or may not have typical features of urinary calculi (pain, hematuria, infection). In some, the diagnosis is made following family studies or during investigation of crystalluria. Very rarely, these diseases present with oliguric acute renal failure, either due to bilateral obstructive stones or to crystal nephropathy, which can exceptionally occur during the neonatal period [16–18]. HPRT deficiency is an X-linked disease that encompasses a large spectrum of neurological and behavioral features, varying from severe early onset neurological symptoms, to asymptomatic hyperuricemic adults [19]. The most severe phenotype corresponds to Lesch-Nyhan syndrome that is characterized by cerebral palsy, choreoathetosis, pyramidal signs, self-mutilation, and undetectable HPRT activity [16, 19]. An intermediate form is represented by subjects with various degrees of neurological involvement, but no self-injurious behavior and residual HPRT activity [19]. HPRT-related hyperuricemia refers to subjects presenting only symptoms secondary to hyperuricemia that have enzymatic activities above 10% [19]. The pathogenesis of neurological and behavioral symptoms in these diseases remains unclear. Very rarely, heterozygous females can manifest severe neurological deterioration [20]. In both APRT and HGPRT deficiencies, urinary urate/creatinine ratio is normal. Plasma uric acid is normal in APRT deficiency, but is markedly elevated in HGPRT deficiency [13]. Both conditions are treated with a high fluid intake, restriction of dietary purines, and allopurinol, which inhibits xanthine oxidase. Rasburicase, a urate oxidase enzyme, has also been used to control hyperuricemia in Lesch-Nyhan nephropathy [17]. Neurological complications have been successfully improved using botulinum toxin injections, oral or intrathecal baclofen, or gabapentin (89).

Diseases of De Novo Purine Biosynthesis

Phosphoribosyl pyrophosphate synthetase I superactivity is a X-linked defect of purine nucleotide biosynthesis, secondary to mutations in the PRPS1 gene, resulting in increased enzyme activity [21]. Patients usually develop urolithiasis and have dysmorphic features in addition to neurological symptoms, including mental retardation, ataxia, developmental delay, and sensorineural deafness [21].

Diseases of Purine Breakdown

Defects of xanthine oxidase dehydrogenase (XOD) include type 1 xanthinuria, caused by isolated XOD deficiency, type 2 xanthinuria, due to deficiencies of both XOD and aldehyde oxidase (AOX), and molybdenum cofactor deficiency, resulting in combined deficiencies of XOD, AOX, and sulfite oxidase [21, 22]. Patients with type 1 and type 2 xanthinuria are clinically similar, but can be distinguished by the ability of type I patients to metabolize allopurinol, while those with type 2 xanthinuria cannot. Hereditary xanthinuria is characterized by very low urinary and plasma concentrations of uric acid and high urinary xanthine excretion, leading to urolithiasis. Many patients with xanthinuria are asymptomatic and are diagnosed after discovering hypouricemia. Rarely, patients develop acute renal failure, or myositis, due to excessive tissue deposition of xanthine [21, 22].

Diseases of De Novo Pyrimidine Synthesis

Among various diseases of pyrimidine metabolism, hereditary orotic aciduria type 1, caused by mutations in the uridine monophosphate synthase gene (UMPS), can present with urolithiasis. Patients often have anemia, which can be megaloblastic or microcytic, unresponsive to vitamin B12, folic acid, iron, or pyridoxine. They are diagnosed by detecting large amounts of orotic acid in their urine. Anemia and urinary orotic acid excretion are corrected by pyrimidine administration [21, 23].

Inherited Defects of Uric Acid Transport

Two rare diseases of uric acid transport cause hereditary renal hypouricemia that is characterized by impaired uric acid reabsorption in proximal tubules. Most patients are asymptomatic and are diagnosed by chance after noticing low serum uric acid levels. Patients may develop urolithiasis and/or exercise-induced acute renal failure in about 10% of cases. Renal hypouricemia type 1 is caused by homozygous or compound heterozygous mutations in the SLC22A12 gene [24, 25], whereas renal hypouricemia type 2 is caused by homozygous or compound heterozygous mutations in the SLC2A9 gene [24, 26]. Urinary alkalization with citrate compounds and hydration are effective in preventing stone formation. Exercise induced acute renal failure is usually self-limiting, without the need of dialysis. Allopurinol has been used as preventive therapy, but evidences of efficacy are lacking [24].

Lecithin-Cholesterol Acyl Transferase Deficiency

Lecithin-cholesterol acyl transferase (LCAT) is an enzyme required for the esterification of cholesterol with unsaturated fatty acid derived from lecithin. Patients with LCAT deficiency accumulate unesterified cholesterol and phosphatidylcholine in plasma and tissues [27]. Symptoms related to tissue accumulation develop during childhood and include grayish corneal opacities, hemolytic anemia and proteinuria, sometimes in the nephrotic range, leading to progressive kidney failure during adulthood [27, 28]. Tendon xanthomata and atherosclerosis have also been described [28]. Biochemically, LCAT deficiency is characterized by low total cholesterol concentrations, variable triglyceride levels, and abnormalities of lipoprotein structure and composition. Renal pathology shows mesangial hypercellularity and expansion, with foamy lipid depositions, vacuolization of the glomerular basement membrane, arteriolar narrowing secondary to intimal thickening and subendothelial lipid deposits [28]. In an Italian cohort of 18 patients, the median age at end-stage kidney disease was 46 years; the disease recurred in transplanted kidneys after approximately 10 years [29].

Lysinuric Protein Intolerance

Lysinuric protein intolerance (LPI) is an autosomal recessive defect of cationic amino acid (lysine, arginine, and ornithine) transport at the basolateral membrane of intestinal and renal tubular cells [30]. In addition, transport defects are also present in nonpolarized cells such as lymphocytes and macrophages [30]. The disease is caused by mutations in the SLC7A7 gene, which encodes for the y^+LAT1 protein; y^+LAT1 forms heterodimers with the protein 4F2hc, which is required for the expression of the transporter at the cell surface [31]. A founder effect explains high incidence of LPI in Finland (1/60,000 births) and to a lesser extent in Southern Italy and Northern Japan. Sporadic cases have been described worldwide. Most, but not all symptoms of LPI are related to derangements of the urea cycle. For long, the disease was considered to represent a benign condition if patients were treated with low-protein diet and citrulline. More careful analysis of LPI patients, however, has shown that LPI is a multisystemic disease and that a significant proportion of subjects develop complications, even under treatment [30, 32]. There is no overt genotype-phenotype correlation; disease severity varies significantly among patients carrying the same mutations. Symptoms range from nearly normal features to severe protein intolerance, failure to thrive, osteoporosis, hepatosplenomegaly, respiratory failure, chronic kidney disease, and immunological diseases, mainly pulmonary alveolar proteinosis, hemophagocytic lymphohistiocytosis, and immune-mediated glomerulonephritis [30, 32]. From the renal standpoint, the majority of patients have mild proteinuria with or without microscopic hematuria; some may progress to end-stage renal failure [32]. Proximal tubular damage, including overt Fanconi syndrome, may be present [32, 33]. In a review of 39 patients aged 1–62 years, 74% had proteinuria, 38% hematuria, 36% hypertension, 38% elevated plasma creatinine, and 4 patients required dialysis [34]. Renal biopsies show diverse pictures, including membranous glomerulonephritis, proliferative glomerulonephritis with immune complex deposits, tubulointerstitial changes, peritubular capillary inflammation, or amyloidosis [35–37]. The most severe cases usually die from pulmonary alveolar proteinosis [32]. High serum levels of inflammatory molecules are frequently observed in these patients, along with elevated ferritin and LDH levels [30, 38].

Typically, patients prefer low-protein foods, but this may lead to nutritional deficiencies [39]. The aim of treatment is to prevent hyperammonemia, while providing sufficient amounts of proteins to allow normal growth. Oral supplementation with L-citrulline primes the urea cycle, preventing postprandial hyperammonemia, and increases protein tolerance [31, 40]. Immune diseases, including glomerular lesions, respond to immunosuppressive treatments, but no systematic studies are available.

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The Kidney in Methylmalonic Acidaemia

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Patrick Forny, Stephanie Grünewald, and
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Abstract

Different inherited metabolic diseases in the cobalamin pathway show a heterogenous kidney involvement. Isolated methylmalonic acidaemias are caused by defects in the genes *MMUT*, *MMAA*, or *MMAB* and are characterized by the accumulation of methylmalonic acid in blood and urine. Possibly due to toxic metabolite effects and a mitochondrial energy deficiency, these disorders result in tubulointerstitial nephritis and progressive kidney failure. Defects elsewhere in the cobalamin pathway, namely, in the genes *MMACHC* and *MTR*, can cause hemolytic uremic syndrome. Raised homocysteine and scarce methionine levels are held accountable to precipitate the kidney pathology. Furthermore, Imerslund-Grasbeck syndrome, caused by proximal cobalamin pathway defects in *CUBN* and *AMN*, is leading to a malabsorption of cobalamin from the gut due to a deficiency of the cubam receptor failing to take up the complex of cobalamin and the intrinsic factor. From a kidney standpoint, these patients suffer from a persistent nonprogressive proteinuria.

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Keywords

Methylmalonic acidaemia · Chronic kidney disease · Tubulointerstitial nephritis · Renal tubular acidosis type 4 · Hemolytic uremic syndrome · Nonprogressive proteinuria

Synonyms

Methylmalonic acidaemia; Methylmalonic acidemia; Methylmalonic aciduria.

Introduction

Methylmalonic acidaemia (MMA) is a group of inherited metabolic diseases, caused by a deficiency of the methylmalonyl-CoA mutase (MMUT) enzyme, either by a direct defect in the *MMUT* gene (OMIM #609058) or a defective synthesis and targeting of the adenosylcobalamin cofactor, which is facilitated by the MMAA and MMAB proteins (*MMAA* gene, OMIM #607481; *MMAB* gene, OMIM #607568) (Fig. 1) [15]. Under physiological conditions the MMUT enzyme converts methylmalonyl-CoA to succinyl-CoA, while in case of the above listed gene defects, a dysfunctional MMUT protein leads to the accumulation of several metabolites, including methylmalonic acid, 2-methylcitric acid, and 3-hydroxypropionate in body fluids, allowing the biochemical diagnosis of the disease based on urine organic acid analysis. Historically, *MMUT* defects are classified as mut^0 in case of an absent response to hydroxocobalamin in the in vitro propionate incorporation assay or as mut^- if propionate incorporation could be enhanced by supplementation of hydroxocobalamin in the cell culture medium [13].

Clinically, MMA presents heterogeneously. Early-onset patients present in the neonatal period with severe metabolic acidosis, leading to lethargy, vomiting, poor feeding, and a sepsis-like clinical picture. In addition to the acidaemia, ketones can be present in urine, and patients might have hyperammonemia, due to secondary inhibition of the urea cycle by toxic metabolites. If untreated, symptoms worsen and progress to coma and eventually death. Milder forms of MMA also exist. Those patients present later in life with developmental delay, hypotonia, seizures, anemia, and failure to thrive. In surviving patients, long-term complications comprise chronic kidney failure and neurological impairment, including movement disorder due to metabolic stroke-like episodes. Patients are generally at risk of metabolic decompensations, which can be triggered by catabolic metabolism due to an infection, diarrhea, vomiting, or prolonged fasting. *MMUT* defects that have significant cobalamin responsiveness in vitro and in vivo (designated mut^-, as compared to mut^0) and MMAA deficiencies seem to be associated with a milder course of the disease [21].

Clinical Symptoms and Biochemical Markers of Kidney Disease in MMA

Renal disease is one of the most devastating long-term complications observed in MMA patients leading to end-stage kidney disease in up to 15% of patients [8]. A European study found that 24% of pediatric and 29% of adult patients have a creatinine clearance of less than 60 mL/min/1.73 m^2 (according to the Schwartz formula) [27]. Another study observed chronic kidney disease (CKD) in almost 50% of patients with a median age of onset of 6.5 years [21]. Of note, mut^0 patients are affected earlier in life (one of the earliest cases of kidney impairment is reported at 18 months of life [41]) compared to *MMAB* patients; in patients with a mut^- or *MMAA* subtype, renal impairment is even milder [17, 21, 22, 27]. These differences were mostly observed in cross-sectional studies, while it is assumed that MMA patients of all subtypes are at risk of developing kidney impairment in the long term [38]. Some studies point toward an association of the severity of CKD with the level of urinary excretion of methylmalonic acid [21], while others do not find quantitative methylmalonic acid excretion to be a predictive factor of glomerular filtration rate (GFR) [1].

Evaluation of kidney function based on creatinine clearance can be unreliable as MMA patients

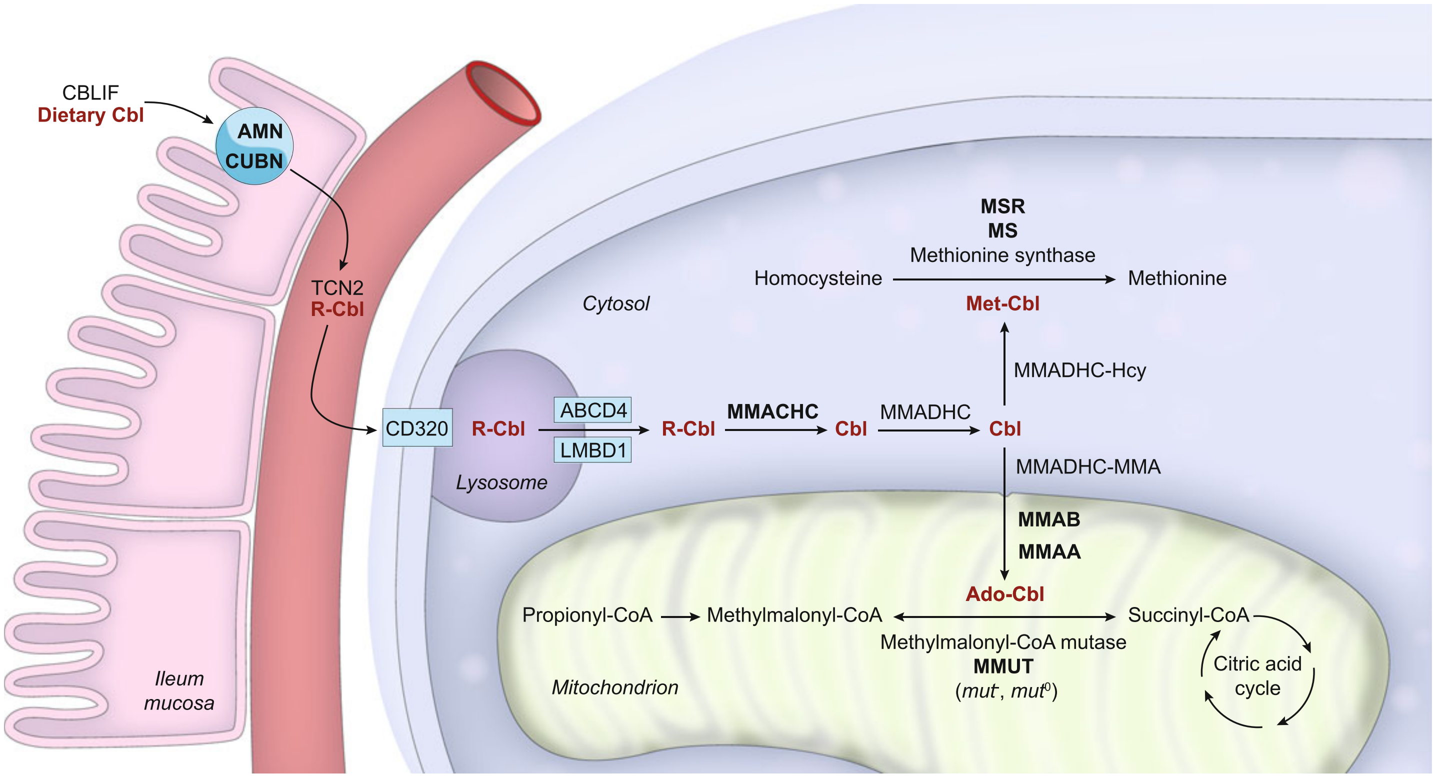
CBLIF
Dietary Cbl
AMN
CUBN
TCN2
R-Cbl
CD320
R-Cbl
ABCD4
LMBD1
Lysosome
Cytosol
R-Cbl
MMACHC
Cbl
MMADHC
Cbl
MSR
MS
Methionine synthase
Homocysteine
Methionine
Met-Cbl
MMADHC-Hcy
MMADHC-MMA
MMAB
MMAA
Ado-Cbl
Propionyl-CoA
Methylmalonyl-CoA
Succinyl-CoA
Methylmalonyl-CoA mutase
MMUT
(mut⁻, mut⁰)
Citric acid cycle
Mitochondrion
Ileum mucosa

Fig. 1 Overview of the cobalamin pathway. After nutritional uptake, cobalamin (Cbl) binds to the intrinsic factor (CBLIF) in the duodenum. Uptake in the terminal ileum is facilitated by the two proteins amnionless (AMN) and cubulin (CUBN), which form the cubam receptor at the apical brush border. Following endocytosis, CBLIF is degraded, and free cobalamin is exported into the bloodstream, where it is bound to haptocorrin (TCN1) and transcobalamin (TCN2). The latter form can be taken up by most body cells after recognition by the transcobalamin receptor (CD320). In the lysosome TCN2 is cleaved off, and the cobalamin molecule is released into the cytosol by the two integral membrane proteins ABCD4 and LMBD1. In the cytosol, MMACHC cleaves off the upper-axial ligand of cobalamin (R group). From there, MMADHC targets cobalamin toward two destinations: (i) cobalamin is used as a cofactor by the cytosolic enzyme methionine synthase (MS) in its methylated form (methylcobalamin, Met-Cbl; MSR, methionine synthase reductase); (ii) in the mitochondrion, adenosylcobalamin (Ado-Cbl) is formed and targeted to methylmalonyl-CoA mutase (MMUT) by MMAB and MMAA. Proteins whose defects are discussed in this chapter are in bold print

have decreased muscle mass [20]. Serum creatinine may be falsely decreased in patients with low muscle mass, leading to an overestimation of kidney function and rendering creatinine poorly predictive of kidney impairment early in disease progression. Estimation of GFR is more accurate using cystatin C, as this biomarker is independent of muscle mass [28]. Cystatin C has been shown, among other factors (height, serum methylmalonic acid concentration), to correlate with kidney growth [28]. Lipocalin-2 has also been used as an alternative biomarker [31, 32], while fibroblast growth factor 21 (FGF21), another recently developed disease biomarker, does not seem to correlate with kidney function [35].

Pathophysiology of Kidney Disease in MMA

Two main pathological renal conditions have been observed in MMA patients: (1) chronic tubulointerstitial nephritis presenting with decreased reabsorption of phosphate, reduced urinary concentrating ability, impaired acid balance, and characterized by interstitial infiltration of inflammatory cells and fibrosis [10, 16, 37] and (2) type 4 renal tubular acidosis with hyperkalemic hyperchloremic metabolic acidosis, hypoaldosteronism, and impaired urinary acidification [37, 49]. Kidney disease in a hypomorphic mouse model of MMA has been shown to cause reduction in urine output, urinary electrolyte losses (sodium, chloride, potassium, calcium, magnesium), and elevated plasma urea [14]. In general, reduced cytochrome C enzyme activity and multiple deficiencies of oxidative phosphorylation as well as structural mitochondrial changes have been noted in MMA models [26, 51]. The exact pathomechanisms remain unsolved, but several detailed studies highlight the effects of toxic metabolites and energy deficiency in the context of mitochondrial impairment as direct consequences of a dysfunctional MMUT enzyme.

Mmut knockout mice are normally nonviable [43]. Manoli *et al.* devised a viable mouse model by expressing a stable *Mmut* transgene under the control of an albumin promoter on a null background to study kidney function in a mammalian model of MMA [32]. The authors found decreased GFR and chronic tubulointerstitial nephritis as observed in patients. More strikingly, they noticed proximal tubule mitochondria with an electron-dense matrix and abnormal cristae morphology associated with abnormal tubular function; the glomeruli and podocyte foot processes appeared normal. Oral treatment of mice with CoQ_{10} or vitamin E led to mitigation of GFR losses and normalization of the plasma biomarker lipocalin-2, suggesting a role of oxidative stress in the pathomechanisms of MMA.

Ruppert *et al.* established an *in vitro* model of tubular epithelial cells derived from patient urine, where they discovered disturbed glycolysis, mitochondrial respiratory chain function, and tricarboxylic acid cycle, together with raised levels of reactive oxygen species [44]. Further, the authors found an increased number of autophagosomes and endoplasmic reticulum stress.

More recently, Luciani *et al.* used patient-derived kidney tubular cells, mouse proximal tubule cells from floxed *Mmut* kidneys, and *mmut*-deficient zebrafish to provide extensive data illustrating that MMUT deficiency induces accumulation of dysfunctional mitochondria which trigger epithelial stress [31]. The authors identified PINK1/Parkin-mediated mitophagy as a key mechanism, which is dysfunctional in MMA, leading to an accumulation of ROS, impaired ATP production, and collapsed mitochondrial membrane potential.

Taken together, current knowledge suggests that effects on mitochondria, rather than elevations in methylmalonic acid per se, are responsible for GFR decrease associated with proximal tubular mitochondrial dysfunction [31, 32]. Interestingly, recent reports have also observed that patients with propionic acidaemia (PA) may develop kidney dysfunction [29]. PA is caused by a defect of propionyl-CoA carboxylase, the enzymatic step just proximal to MMUT. PA patients present with a very similar, although often more severe, phenotype but with milder and more delayed kidney dysfunction [4]. Thus, although recent findings have challenged previous hypothesis suggesting that methylmalonic acid acts as a toxic agent for the kidney [25, 49], the significantly milder kidney phenotype observed in PA suggests that there may still be a pathophysiological role of methylmalonic acid in the development of MMA-associated kidney disease.

Renal-Related Complications in MMA

Some complications observed in MMA patients are secondary to kidney impairment or are at least partly influenced by a dysfunction of the kidney. These symptoms include anemia, arterial hypertension, renal osteodystrophy, and hyperparathyroidism [37]. MMA patients are at risk of low bone density due to chronic acidosis, inadequate nutritional intake of calcium and phosphate (as a consequence of low natural protein diet), and low vitamin D levels (secondary to impaired renal synthesis), leading to hyperparathyroidism and calcium mobilization from the bones [21]. Further, cytopenia of all blood lineages has been described in MMA [3]. Neutropenia and thrombocytopenia can occur chronically or during an acute metabolic decompensation, while anemia is usually a chronic problem possibly due to insufficient production of erythropoietin by the failing kidney.

Treatment Options

Clinical guidelines for MMA provide assistance for the management of MMA patients [4]. The mainstay of MMA therapy consists of a specific diet low in natural protein to limit the intake of precursor amino acids (valine, threonine, isoleucine, methionine) which funnel into the MMUT pathway. This regimen can be supplemented with precursor-free amino acid mixtures. Further, levocarnitine is given to conjugate and excrete toxic intermediary metabolites, such as propionyl-CoA and methylmalonyl-CoA, and to replenish lowered free carnitine levels. All patients should be evaluated clinically for cobalamin responsiveness by intramuscular administration of hydroxocobalamin [15].

Due to the lack of high-grade evidence, guidance on the treatment of kidney dysfunction in MMA is mostly limited to standard treatment strategies. Hemodialysis and peritoneal dialysis have been successfully applied in patients with end-stage renal disease [12, 21]. Also, hemodialysis is often used as an emergency therapy to treat hyperammonemia or severe metabolic acidosis during metabolic decompensation and together with other improvements in intensive care medicine is probably one of the reasons explaining decreased morbidity of MMA disease over the last decades [18].

Combined liver and kidney transplantation can effectively treat kidney failure in MMA patients, with good renal outcome up to 10 years post-transplant [9, 11, 36, 48, 50]. Isolated kidney transplant has been performed as well and found to improve kidney function for a similar amount of years in some reports [5, 7, 30], but in other cases, reoccurrence of renal dysfunction and kidney failure has been reported after transplantation. Isolated liver transplantation appears to preserve kidney function only in some cases, and progressive kidney failure has been described in several cases post-liver transplant [9, 24, 40, 46]. There are no reports of improvement of an already dysfunctional kidney after liver transplantation [45]. Overall, organ transplantation in MMA remains controversial, as detailed effects on the manifold of outcome parameters have not been studied in the long term, and especially neurological complications do not seem to be cured/avoided by this mode of therapy [34, 39].

There is still a considerable unmet need for effective therapies for MMA patients. Future developments might include treatment modalities based on genome editing or the usage of messenger RNA. A specific lipid nanoparticle formulation in combination with an mRNA expression cassette has been successfully used to treat *Mmut*-deficient mice [2]. For AAV-based approaches, novel virus capsids are currently being evaluated.

Other Defects in the Cobalamin Metabolism

In addition to the above-described forms of isolated methylmalonic acidaemia, further genetic defects in the cobalamin pathways exist [23].

Among others, defects in the *MMACHC* (OMIM #609831) gene cause methylmalonic aciduria and homocystinuria. Defects in the *MTR* (OMIM #156570) and *MTRR* (OMIM #602568) genes lead to a deficiency of the methionine synthase (MS) enzyme and the biochemical phenotype of isolated homocystinuria. In both, MMACHC and MS deficiency, hemolytic uremic syndrome has been observed as a renal complication. The pathomechanisms are different to the ones discussed above for isolated MMA and include (i) a toxic effect of homocysteine and (ii) the lack of the downstream metabolite methionine. Homocysteine might damage the glomerular endothelium, leading to a pro-thrombotic milieu secondary to induced tissue plasminogen activator, activated inflammatory pathways, and accumulation of intracellular radicals [19, 37, 42, 47]. Methionine deficiency might be partly responsible for the type of vessels which are affected [33].

Lastly, gene defects further proximal in the pathway (*CUBN*, OMIM #602997; *AMN*, OMIM #605799) lead to a defective absorption of the cobalamin-intrinsic factor complex and to the clinical picture of Imerslund-Grasbeck syndrome. These patients present with decreased serum cobalamin, low-molecular-weight proteinuria, and mild clinical symptoms (failure to thrive, loss of appetite, fatigue, lethargy) [6].

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The Kidney in Mitochondrial Diseases

33

Fatih Ozaltin, Leonardo Salviati, and Shamima Rahman

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Abstract

Mitochondria are small dynamic intracellular organelles with critical roles in energy generation and other aspects of cellular homeostasis. Mitochondrial disorders, which are estimated to have a population prevalence of at least 1 in 4300, are monogenic diseases associated with dysfunction of the oxidative phosphorylation system, mitochondrial ultrastructure and dynamics, production of cofactors and vitamins, or other metabolic processes within the mitochondrion. Renal manifestations are a relatively frequent complication of mitochondrial disease and include Fanconi-type tubulopathy, chronic tubulointerstitial nephritis, steroid-resistant nephrotic syndrome, and cystic glomerular disease. Isolated kidney disease is a rare presentation of mitochondrial disease, although it does occur, particularly in some types of coenzyme Q_{10} (CoQ_{10}) deficiency. More frequently, renal involvement is part of a complex multisystemic disease. The genetics of mitochondrial disease is complex and includes mutations of the intrinsic mitochondrial DNA (mtDNA) as well as more than 300 nuclear-encoded Mendelian disorders affecting mitochondrial function. The most frequent causes of mitochondrial nephropathy are point mutations and large-scale rearrangements of the mtDNA and autosomal recessive disorders of CoQ_{10} biosynthesis. Increasingly, exome and genome sequencing of large cohorts is leading to the identification of novel genetic causes of mitochondrial kidney disease, including disorders of mtDNA maintenance, mitochondrial translation, mitochondrial dynamics, and biosynthesis of membrane lipids and iron-sulfur clusters. Diagnosis is challenging, as there may be few clues from renal or muscle biopsy and biochemical testing, and the approach to diagnosis increasingly involves genome-wide next generation sequencing.

Keywords

Mitochondria · Kidney · Mitochondrial DNA · Nuclear DNA encoding mitochondrial proteins · Oxidative phosphorylation · Coenzyme Q_{10} deficiency

Introduction

Mitochondria are small dynamic intracellular organelles that play a central role in cellular homeostasis. Their principal function is the generation of ATP through oxidative phosphorylation (OXPHOS). The OXPHOS system comprises five enzymatic complexes and two electron carriers, cytochrome *c* and coenzyme Q_{10} (CoQ_{10}). Mitochondria host several other metabolic pathways and control intracellular calcium homeostasis and the intrinsic apoptotic pathway [1, 2]. Mitochondria possess their own genome (the mitochondrial DNA, mtDNA) that is a 16,569 base pairs long circular molecule, lacks introns, and has its own genetic code (different from the universal code) for protein synthesis. However, the vast majority of genes needed for mitochondrial function are encoded in the nucleus. MtDNA is present in multiple copies per mitochondrion (and in hundreds of copies in each cell), and its transmission does not follow the laws of Mendel. Firstly, mtDNA is inherited only from the mother (**Maternal Inheritance**). Both males and females may be affected by a mtDNA-related disorder, but only females will transmit it to their offspring. Secondly, wild type and mutant mtDNA molecules coexist in different proportions within cells of the same tissue, or within different tissues of the same individual (**Heteroplasmy**). Thirdly, mutations must affect a critical proportion of the total mtDNA in order to cause a biochemical or clinical phenotype (**Threshold Effect**). Fourthly, mtDNA molecules segregate randomly in daughter cells at cell division (**Random Drift**). Tissues with high cell turnover, such as the bone marrow, are more likely to be relatively spared because cells with higher mutation loads are cleared during proliferation. Conversely, postmitotic cells, such as neurons or renal cells, tend to accumulate mutations and are more likely to develop mitochondrial dysfunction [3].

MtDNA encodes 37 genes, including 13 OXPHOS subunits, and the 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (rRNAs) required for mitochondrial protein synthesis. OXPHOS complexes contain more than 80 subunits in total. Hundreds of other proteins are required for mtDNA maintenance, replication, transcription, and for mitochondrial protein synthesis and cofactor biosynthesis, all of which are encoded by nuclear genes, synthesized in the cytosol and then imported into mitochondria. Hence, OXPHOS biogenesis requires the coordinated action of two distinct genomes, and its dysfunction may result from mutations in either mitochondrial or nuclear genes (Fig. 1) [4].

OXPHOS dysfunction causes a wide spectrum of clinical phenotypes that may present at any age and may affect virtually any tissue, although those that rely the most on aerobic metabolism (nervous system, skeletal and cardiac muscle) are usually most severely affected. In recent years, it has become evident that renal involvement is not at all rare in these disorders.

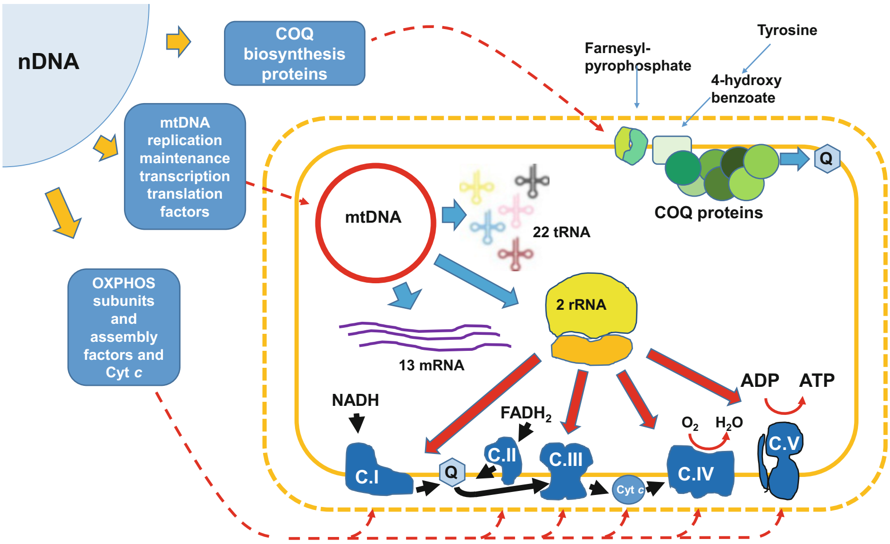

Fig. 1 Relationships between the nuclear (nDNA) and mitochondrial (mtDNA) genomes in the biogenesis of the mitochondrial oxidative phosphorylation (OXPHOS) system. mtDNA provides 13 mRNAs encoding OXPHOS structural subunits [7 of complex I (C.I), 1 of C.III, 3 of C.IV, and 2 of C.V) and the ribosomal RNA (rRNA) and transfer RNA (tRNA) molecules required for mitochondrial protein synthesis. nDNA provides more than 70 other subunits of OXPHOS complexes, as well as cytochrome *c* (Cyt *c*), assembly factors, and all other factors required for mtDNA replication, maintenance, transcription, and translation. nDNA also encodes the proteins required for Coenzyme Q_{10} (COQ, Q) biosynthesis. The black arrows depict the flow of electrons between the different complexes and electron carriers

Mitochondrial DNA Defects Associated with Kidney Disorders

Primary mtDNA defects comprise point mutations (the most frequent is the m.3243A>G in *MT-TL1*-encoding tRNA$^{Leu(UUR)}$, present in 1 in 400 of the population) and gross rearrangements (essentially deletions) which typically span from 2 to 8 kb in size [5]. Point mutations may affect the 13 structural subunits, and tRNA or rRNA genes. Mutations of tRNAs and rRNAs impair mitochondrial protein synthesis as a whole. This is true also for large deletions, since they always include at least one tRNA gene (Table 1).

Because some residual OXPHOS activity is essential for development, pathogenic mtDNA variants are usually heteroplasmic, i.e., a certain proportion of wild type mtDNA molecules are still present. Conversely, polymorphisms are usually homoplasmic (i.e., they affect all mtDNA molecules in an individual), although a few bona fide homoplasmic mtDNA-pathogenic variants causing renal dysfunction have been reported (and confirmed by subsequent reports or functional studies) such as the m.616T>C in *MT-TF* encoding tRNA^{Phe} (Table 1) [4, 6].

MtDNA mutations have been associated with proximal or distal tubular dysfunction, and with glomerulopathies; more rare phenotypes are tubulointerstitial nephritis (TIN) and cystic glomerular disease (Table 1) [7]. Renal involvement can be an isolated manifestation or, more frequently, a part of a complex syndrome, affecting other tissues. The most common mtDNA-related syndromes associated with renal involvement are Kearns-Sayre (KSS) and Pearson syndromes, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), and maternally inherited diabetes and deafness (MIDD), but there are also patients whose symptoms do not fit into any of these syndromic diagnoses. In some cases, renal manifestations are the presenting symptom, and extrarenal involvement may become evident only after months or years (leading to significant diagnostic delay), and in other cases the renal disease remains the only manifestation [8, 9]. The complexity of inheritance and transmission of mtDNA explains only part of the phenotypic variability. Genotype phenotype correlations are still not clearly understood. Our knowledge of the pathophysiology of these conditions is still limited, and we lack animal models for these diseases, although the recent discovery of enzymes capable of mtDNA base editing may finally allow the generation of mouse models harbouring specific mtDNA mutations [10].

The proximal and distal tubules have high energetic demands and are rich in mitochondria. Tubular involvement is observed frequently in mitochondrial disorders and is linked to ATP deficiency. Proximal tubular dysfunction may range in severity from complete Fanconi syndrome with low-molecular-weight proteinuria, to partial defects, such as isolated renal tubular acidosis, glycosuria, aminoaciduria, a Bartter-like phenotype, or even isolated hypermagnesuria. In the majority of cases, these manifestations are seen in the context of a multisystemic disorder (such as KSS). Most of these patients harbored deletions of mtDNA, more rarely point mutations such as the m.8344A>G in *MT-TK* encoding tRNA^{Lys} which has been associated with aminoaciduria (Table 1) [11].

Several patients have been reported in the literature with dysfunction of the distal convoluted tubule, presenting with specific electrolyte alterations, most frequently hypomagnesaemia and hypokalemia. Again, patients displayed in most cases a multisystemic phenotype and harbored mtDNA deletions.

The glomerulus relies less on OXPHOS since anaerobic glycolysis maintains glomerular filtration independent of mitochondrial metabolism [12]. Nevertheless, glomerular involvement is often observed in MELAS and MIDD, associated with the m.3243A>G mutation [13]. Clinical manifestations may range from proteinuria, often without haematuria, to overt nephrotic syndrome, with progression to renal failure. The histological picture is usually characterized by focal and segmental glomerulosclerosis. These patients are often misdiagnosed with Alport syndrome due to coexistence of sensorineural hearing loss (SNHL). However, absence of haematuria and typical histopathological features characterizing

Table 1 Mitochondrial kidney disease: examples of molecular mechanisms

Molecular defect	Gene(s)	Inheritance	Renal manifestations	Other clinical phenotypes
mtDNA-encoded				
Large-scale rearrangements of mtDNA	Multiple	Sporadic	Fanconi-type tubulopathy, tubulointerstitial nephritis	Pearson and Kearns-Sayre syndromes, PEO
mtDNA point mutations (selected examples)	*MT-TL1*	Maternal	FSGS	MELAS, MIDD
	MT-TK	Maternal	Tubulointerstitial nephritis	MERRF
	MT-TF	Maternal/sporadic	Tubulointerstitial nephritis	Encephalomyopathy
	MT-ND5	Maternal/sporadic	Glomerulocystic disease	Leigh syndrome, MELAS
Nuclear-encoded				
Coenzyme Q_{10} biosynthesis	*COQ2, COQ6, COQ8B, PDSS1, PDSS2*	AR	Steroid resistant nephrotic syndrome	Seizures, ataxia, hearing loss, and multisystem disease
	COQ9	AR	Tubulopathy	Encephalomyopathy, HCM
Complex I assembly	*NDUFAF2*	AR	Renal tubular acidosis	Leigh syndrome
Complex III subunits	*UQCC2, UQCRC2*	AR	Tubular dysfunction	Neonatal lactic acidosis
Complex III assembly	*BCS1L*	AR	Proximal tubulopathy	GRACILE, encephalopathy, and hepatopathy
Complex IV assembly	*COX10*	AR	Tubulopathy	Leigh syndrome
	SURF1		Distal renal tubular acidosis	Leigh syndrome
Complex V subunit	*ATP5A1*	AR	Renal cysts	Fatal neonatal encephalopathy
Complex V assembly	*ATPAF2*	AR	Hypoplastic kidneys	Cerebral and skeletal abnormalities, lactic acidosis
	TMEM70	AR	Proximal tubulopathy	HCM, lactic acidosis, hyperammonaemia, and 3MGA
mtDNA maintenance	*RRM2B, DGUOK, TK2, SUCLA2, MPV17*	AR	Proximal tubulopathy	Mitochondrial DNA depletion syndrome (hepatocerebral, myopathic, and encephalomyopathic)
	POLG	AR	Renal tubular acidosis	Epilepsy, ataxia, and PEO
Mitochondrial tRNA aminoacylation	*SARS2*	AR	Tubulo-interstitial disease with salt wasting and hypomagnesaemia	HUPRA
Mitochondrial ribosome	*MRPS16, MRPS22, MRPS34, MRPL44*	AR	Tubulopathy	HCM, encephalomyopathy
Mitochondrial translation	*TFSM*	AR	Tubulopathy	IUGR, hepatic insufficiency, hypotonia
	RMND1	AR	End-stage kidney disease	Multisystem disease
Iron-sulfur cluster biogenesis	*NFS1*	AR	End-stage kidney disease	Multisystem disease

(continued)

Table 1 (continued)

Molecular defect	Gene(s)	Inheritance	Renal manifestations	Other clinical phenotypes
Membrane lipid remodeling	*SERAC1*	AR	Tubular dysfunction	MEGDEL
Mitochondrial homeostasis	*CLPB*	AR	Nephrocalcinosis	Cataracts, 3MGA, and multisystem disease
Mitochondrial chaperone	*TRAP1*	AR	CAKUT	VACTERL association
Other disease mechanisms	*C1QBP*	AR	Congenital nephrosis	HCM, multisystem disease

Key: 3MGA, 3-methylglutaconic aciduria; AR, autosomal recessive; CAKUT, congenital abnormalities of the kidney and urinary tract; FSGS =; GRACILE, growth retardation, amino aciduria, cholestasis, iron overload, lactic acidosis, and early death; HCM, hypertrophic cardiomyopathy; HUPRA, hyperuricaemia, pulmonary hypertension, renal failure, and alkalosis; IUGR, intrauterine growth restriction; MEGDEL, 3-methylglutaconic aciduria, deafness, and Leigh-like encephalomyopathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibres; MIDD, maternally inherited diabetes-deafness; and PEO, progressive external ophthalmoplegia

Alport syndrome facilitates ascertainment of the correct diagnosis. Other mtDNA point mutations, and occasionally deletions, may present with glomerular involvement [5]. The pathogenesis of glomerular dysfunction is still not clear. In general, defects in OXPHOS produce two major effects: a decrease in ATP production and an increase in generation of reactive oxygen species, each of which has been implicated in the pathogenesis of the renal manifestations of mitochondrial disease. In addition, defects in electron carriers such as CoQ_{10} and cytochrome *c* also affect apoptosis as they have important roles in this process by modulating the mitochondrial permeability transition pore, which acts as a gating channel for apoptosis [1, 9].

Chronic TIN is a histopathological rather than clinical diagnosis, and it is characterized by the presence of tubulointerstitial inflammatory cell infiltrates and interstitial edema. Clinically, patients present with nonspecific symptoms such as renal failure and low molecular weight proteinuria. TIN has been associated with mtDNA deletions, and with several point mutations, especially in *MT-TF*. Finally, glomerulocystic disease has been associated with a mutation in the *MT-ND5* gene (Table 1) [5].

Nuclear-Encoded Mitochondrial Kidney Disorders

Coenzyme Q_{10} Deficiencies and Related Nephropathies

Primary CoQ_{10} deficiencies are unique among mitochondrial disorders, as they are amenable to treatment with exogenous supplementation of CoQ_{10}. They therefore deserve special emphasis since early diagnosis and CoQ_{10} supplementation are essential to prevent sustained organ damage. In common with all mitochondrial diseases, this group of autosomal recessive disorders is clinically and genetically heterogeneous. Kidney involvement may be isolated or occur as part of a multisystemic disease [9]. In many affected individuals, progressive kidney disease is characterized by proteinuria or steroid-resistant nephrotic syndrome (SRNS) with or without haematuria clinically and by focal segmental glomerulosclerosis (FSGS) lesions, particularly its collapsing variants and rarely diffuse mesangial sclerosis, histopathologically. Light microscopic findings are not specific; however, although not always visible in electron microscopy, high numbers of dysmorphic mitochondria in the cytoplasm of podocytes might be indicative of an underlying mitochondrial disease [1]. After the identification of the first link between CoQ_{10} deficiency and renal disease in the year 2000 [14], several genes involving endogenous CoQ_{10} biosynthesis including *COQ2, COQ6, COQ8B, PDSS1, PDSS2,* and *COQ9* have been discovered to cause mainly glomerular but rarely tubular disorders when mutated and altogether are responsible for 1% of SRNS (Table 1) [9, 15].

COQ2 Nephropathy

COQ2-encoding 4-para-hydroxybenzoate polyprenyltransferase, an enzyme that catalyzes the second step in the mitochondrial biosynthesis of endogenous CoQ_{10}, was the first gene defect linked to primary CoQ_{10} deficiency [16]. It is a rare disorder, with 27 patients with biallelic *COQ2* variants reported in the literature to date [17, 18]. The disease manifests with three clinical presentations: (a) isolated nephrotic syndrome, (b) cerebrorenal disease, and (c) multisystemic disease. Reported extrarenal findings include progressive ataxia, generalized amyotrophy, retinitis pigmentosa, nystagmus, bilateral SNHL, psychomotor delay or progressive psychomotor regression, optic atrophy, hypotonia, seizure, acute respiratory distress, hypertrophic cardiomyopathy, liver failure, anaemia, pancytopaenia, insulin-dependent diabetes mellitus, and metabolic acidosis [9]. Onset is usually before the age of 2.5 years, but adolescent onset SRNS has also been reported [19]. In the initial reports of COQ2 deficiency, CoQ_{10} supplementation was suggested to improve neurological symptoms but not the renal findings. However, these initial patients had advanced kidney disease at the time of commencing CoQ_{10} supplementation, and subsequent studies showed beneficial effects of CoQ_{10} supplementation on proteinuria if started immediately after onset of renal symptoms [17, 18, 20] but no improvement of progressive encephalopathy [17].

COQ6 Nephropathy

COQ6 encodes a monooxygenase enzyme that catalyzes the C5 hydroxylation step of the quinone ring during endogenous CoQ_{10} biosynthesis [9]. To date, 21 patients with biallelic *COQ6* variants have been reported. The main clinical presentation is progressive SRNS resulting in end stage kidney disease (ESKD) and SNHL. The median age of diagnosis is 1.2 (range 0.2–7) years [21]. Extrarenal findings including seizure, white matter abnormalities, ataxia, facial dysmorphism, growth retardation, muscle weakness, exotrophia, nystagmus, and bilateral optic nerve atrophy have been reported [22]. Untreated, ESKD develops between 0.4–9 years of age. Exogenous CoQ_{10} supplementation may be beneficial and should be considered for protecting renal function; however, its beneficial effect on SNHL and other extrarenal findings does not seem to be promising [9, 21].

PDSS1 and PDSS2 Nephropathies

PDSS1 encodes decaprenyl-diphosphate synthase subunit 1, which is required for the synthesis of the polyisoprenoid chain, while *PDSS2* encodes decaprenyl-diphosphate synthase subunit 2, which is required for the elongation of the prenyl side chain during biosynthesis of CoQ_{10}. These disorders are extremely rare, manifest as infantile-onset multisystemic disease, and are generally fatal despite exogenous CoQ_{10} therapy. In 2000, three siblings with similar symptoms but different degrees of severity characterized by nephrotic syndrome, ataxia, dystonia, muscle weakness, retinitis pigmentosa, SNHL, and cardiomyopathy were reported in whom *PDSS2* mutation as an underlying etiology was subsequently identified [14, 23]. Since then, *PDSS2*-related disorders have been reported in four further patients, all with SRNS [15, 24, 25]. Associated findings in these patients include neonatal pneumonia, hypotonia, seizure, changes in the basal ganglia consistent with Leigh syndrome, encephalomyopathy, hypertrophic cardiomyopathy, SNHL, and retinitis pigmentosa. *PDSS1*-related nephropathy has been described in only one patient with infantile SRNS, developmental delay, leukoencephalopathy, lactate peak, and faltering growth [26].

COQ8B Nephropathy

COQ8B (previously known as *ADCK4*) encodes an atypical kinase that interacts with components of the CoQ_{10} biosynthesis pathway. Patients with *COQ8B* mutations have reduced cellular CoQ_{10} content [27]. Among the CoQ_{10}-related nephropathies, recessive mutations in *COQ8B* account for the highest number of patients and constitute an important differential diagnosis in adolescents with SRNS/FSGS and/or chronic kidney disease (CKD) of unknown origin [28, 29]. The renal phenotype of *COQ8B* disease is characterized by an insidious onset at adolescence with mild-to-moderate proteinuria and absence of apparent edema in the majority of cases. As a consequence of the oligosymptomatic early course, advanced CKD is often present at the time of diagnosis. Haematuria may be present at presentation in 25% of patients, and FSGS is the main histopathological diagnosis [28]. In contrast to other CoQ_{10} deficiencies, *COQ8B* disease typically manifests as an isolated nephropathy with only occasional extrarenal symptoms, which have been described in only a few patients and include seizure, intellectual impairment, defects of visual field, retinitis pigmentosa, agoraphobia, hypertrophic cardiomyopathy, autism, and pulmonary hypertension [28, 29]. Although clinical presentation is not usually seen until the adolescent period, asymptomatic proteinuria may actually be present at any age between 4.4 and 39 years [29]. *COQ8B*-related nephropathies are among the CoQ_{10} deficiencies that appear to benefit most from exogenous CoQ_{10} supplementation. In animal models, knocking out *Coq8b* resulted in reduced podocyte motility in vitro, which could be reversed by adding CoQ_{10} to the culture medium [27]. In humans, exogenous CoQ_{10} supplementation has been shown to decrease proteinuria while preserving glomerular filtration rate [27–30]. In view of its insidious nature, screening of apparently asymptomatic individuals for early diagnosis and avoidance of progressive renal disease by early initiation of CoQ_{10} treatment are recommended in families with at least one affected individual with *COQ8B* nephropathy [29, 30].

COQ9 Nephropathy

COQ9 is necessary for the stability and function of COQ7. Mutations in *COQ9* are extremely rare and have been reported in only a few individuals. Reported findings include neonatal lactic acidosis, intractable seizures, global developmental delay, microcephaly, dystonia, left ventricular hypertrophy, Leigh-like syndrome, truncal hypotonia, dysmorphic features, cardiomyopathy, tubulopathy, abnormal appearing kidneys, and cystic kidneys [9, 31]. Limited clinical benefit of exogenous CoQ_{10} supplementation was observed in these patients.

Other Nuclear-Encoded Mitochondrial Disorders Associated with Renal Involvement

Approaching 1500 nuclear-encoded proteins are needed for mitochondrial function, and more than 350 of these have now been linked to mitochondrial disease [2]. In addition to the CoQ_{10} biosynthesis disorders described above, pathogenic variants in dozens of nuclear genes have been reported to cause significant renal dysfunction, usually in the context of complex multisystem disorders presenting in childhood [32]. Renal manifestations of these gene defects include tubulopathy, ESKD, nephrocalcinosis, and congenital kidney abnormalities. Detailed discussion of all of these gene defects is beyond the scope of this chapter, but we describe the pathological mechanisms of nuclear-encoded mitochondrial disease and some of the more common disorders linked to renal manifestations.

Disorders of OXPHOS Subunits and Assembly Factors

Mutations of nuclear-encoded structural OXPHOS proteins have rarely been reported to cause renal disease. Exceptions include two complex III subunits, UQCC2 and UQCRC2, mutations of which have been linked to tubular dysfunction and neonatal-onset lactic acidosis [33, 34]. Mutations of a complex V subunit ATP5A1 caused a fatal neonatal encephalopathy with renal cysts [35].

The complex nature of the OXPHOS enzymes means that their assembly is an intricate process involving many assembly factors. Renal tubulopathy has been documented in defects of the assembly of complexes I, III, IV, and V (Table 1). The most prevalent of these disorders is BCS1L deficiency, a disorder of complex III assembly that has been linked to a wide clinical spectrum. The most severe phenotype of BCS1L deficiency is GRACILE (Growth Retardation, Aminoaciduria, Cholestasis, Iron overload, Lactic acidosis, and Early death) syndrome, but tubulopathy is a frequent feature in patients with *BCS1L* mutations leading to other clinical presentations, typically including encephalopathy and hepatopathy [36]. Mutations of two factors needed for complex V assembly have also been reported to cause renal manifestations. An infant with ATPAF2 deficiency had hypoplastic kidneys [37], while renal involvement was present in 34% of a large cohort of children with biallelic variants in *TMEM70*, in association with neonatal onset cardiomyopathy, lactic acidosis, hyperammonaemia, and 3-methylglutaconic aciduria, with a progressive neurological course [38].

Mitochondrial DNA Maintenance Defects

Disorders of mtDNA maintenance may be divided broadly into defects of the mitochondrial replication apparatus and defects of nucleoside supply for mtDNA synthesis. Mutations of *POLG* encoding the catalytic subunit of DNA polymerase gamma, the only enzyme able to replicate mtDNA, are the most prevalent single gene cause of mitochondrial disease. *POLG* mutations result in mtDNA depletion and/or multiple mtDNA deletions and present with neurological disease, usually epilepsy, ataxia, or ophthalmoplegia but can occasionally be associated with renal tubular acidosis [39]. Two defects of mitochondrial nucleoside salvage, DGUOK and MPV17 deficiencies, cause mtDNA depletion syndromes manifesting with liver failure, encephalomyopathy, and renal tubulopathy [40, 41]. A third disorder, RRM2B deficiency, is a defect of cytosolic de novo nucleoside synthesis. Infantile-onset RRM2B deficiency now appears to be a relatively homogeneous condition with neonatal onset muscle weakness leading to feeding difficulties and respiratory failure, frequently associated with SNHL and gastrointestinal manifestations, and with renal tubulopathy reported in 55% of cases [42].

Disorders of Mitochondrial Translation

The burgeoning group of disorders of mitochondrial gene expression, which includes defects of mitochondrial RNA modification, aminoacyl tRNA synthetases, ribosomal proteins, and translation elongation factors, is frequently accompanied by renal manifestations (Table 1). Mutations of *SARS2*, encoding the mitochondrial aminoacyl tRNA synthetase for serine, were initially linked to a syndrome of hyperuricemia, pulmonary hypertension, and renal failure in infancy with alkalosis (HUPRA) [43] but more recently have been reported to cause spastic paraparesis without renal involvement. Defects of several other mitochondrial aminoacyl tRNA synthetases are associated with tubular dysfunction, as are mutations of four mitochondrial ribosomal proteins (Table 1). One of the most frequently reported mitochondrial translation defects resulting in ESKD is RMND1 deficiency, which initially manifests with a neonatal pseudohypoaldosteronism type picture associated with SNHL, with later progressive renal disease associated with myopathy and lactic acidosis [44].

Defects of Mitochondrial Lipid Membranes and Dynamics

Another growing subgroup of mitochondrial disorders includes defects of proteins needed for the synthesis of mitochondrial membrane lipids and for mitochondrial dynamics (fission and fusion). SERAC1 deficiency, a defect of mitochondrial membrane lipid remodelling, causes a syndrome of 3-methylglutaconic aciduria, deafness, and Leigh-like encephalomyopathy (MEGDEL). Impaired tubular function was noted in the neonatal period in 12% (8/66) of cases in one large series of patients with *SERAC1* mutations, and was a transient problem in most cases, who went on to have a progressive neurodegenerative disorder dominated by dystonia (Table 1) [45].

Disorder of Iron-Sulfur Cluster Biosynthesis

Iron-sulfur (Fe-S) clusters are essential components of several mitochondrial enzyme complexes, and numerous defects of Fe-S cluster biosynthesis have been linked to mitochondrial disease. Examples of Fe-S cluster defects associated with renal disease include NFS1 deficiency, which was reported to cause ESKD in two infants (Table 1) [46].

Other Disease Mechanisms

Novel molecular mechanisms of mitochondrial disease are increasingly recognized, and frequently associated with renal abnormalities. For example, mutations of *TRAP1*, encoding tumor necrosis factor receptor-associated protein 1, a mitochondrial chaperone, have been linked to congenital abnormalities of the kidney and urinary tract (CAKUT) and VACTERL association [47]. Deficiency of CLPB, an enzyme needed for mitochondrial homeostasis, was reported to cause cataracts, renal cysts, and nephrocalcinosis with 3-methylglutaconic aciduria [48] but has also been linked to more complex infantile onset multisystem disorders, occasionally with ESKD [49]. Mutations of *C1QBP*-encoding complement component 1 Q subcomponent-binding protein caused mitochondrial cardiomyopathy with congenital nephrosis in one case (Table 1) [50]. Other disease mechanisms associated with mitochondrial kidney disease include mitochondrial toxicity and disorders of antioxidant defense.

Approach to Diagnosis of Mitochondrial Kidney Disease

The diagnosis of these conditions remains complex. Routine renal histology is usually not specific for mitochondrial diseases. It is possible to perform histochemical studies assessing cytochrome *c* oxidase and succinate dehydrogenase activities on frozen sections, similarly to what is routinely performed on skeletal muscle samples. These tests, however, have never gained popularity, partly due to technical issues, and in part because abnormal histochemical staining patterns are often observed in acquired disorders. Electron microscopy can be useful to exclude other conditions, particularly Alport syndrome. The presence of abnormally shaped mitochondria in podocytes and/or tubular cells, or signs of mitochondrial proliferation, may provide important clues to the diagnosis [51].

Next generation sequencing has revolutionized the diagnosis of Mendelian disorders, including nuclear-encoded mitochondrial disorders. It is now possible to study panels comprising hundreds of genes in a nuclear mitochondrial gene panel, which can also be adapted to capture the mtDNA. Furthermore, reduction in costs is making the analysis of the entire exome or genome a realistic alternative in the routine diagnostic setting.

The identification of causative mtDNA mutations can be more problematic due to heteroplasmy and random drift events. However, next generation sequencing is also an attractive tool for analyzing low-level heteroplasmic mtDNA mutations. The high throughput allows great breadth of coverage (analyzing whole mtDNA genomes across multiple samples) and great depth of coverage (resolution of very low-level variants).

Analysis of DNA extracted from muscle was considered the gold standard, but it requires an invasive procedure. In recent years, analysis of DNA extracted from urinary sediment cells has become popular. It is possible to screen for mtDNA deletions and for the common point mutations, or sequence the entire mitochondrial genome.

Management and Treatment

Individuals with mitochondrial disorders should be managed by a multidisciplinary team depending on the spectrum of renal and extra-renal findings. Early diagnosis is particularly important for CoQ_{10}-related disorders as early supplementation of CoQ_{10} may prevent these individuals from progressive disease. No evidence-based study regarding dosage has been published. Therefore, CoQ_{10} has been given to patients with empirical doses 30–50 mg/kg per day [9, 20, 29, 30]. Since the bioavailability and the correlation between plasma and tissue levels of CoQ_{10} are poor, observing clinical response is the only practical method to monitor the efficacy of therapy. Regular surveillance for and supportive management of extrarenal manifestations is important; such supportive measures may include antiepileptic drugs, hearing aids, cochlear implants, brow suspension for ptosis, pacing for heart block, medical management of cardiomyopathy, blood transfusions in Pearson syndrome, hormone replacement, and appropriate nutritional support.

Cross-References

- ▶ Aminoaciduria and Glycosuria in Children
- ▶ Approach to the Child with Hematuria and/or Proteinuria
- ▶ Clinical Aspects of Genetic Forms of Nephrotic Syndrome
- ▶ Congenital Nephrotic Syndrome
- ▶ Ethical Issues in Pediatric Nephrology
- ▶ Genetic Basis of Nephrotic Syndrome
- ▶ Inherited Diseases of the Glomerular Basement Membrane
- ▶ Inherited Disorders of Renal Magnesium Handling
- ▶ Nephrotic Syndrome: Classification and Evaluation
- ▶ Pediatric Fanconi Syndrome
- ▶ Pediatric Tubulointerstitial Nephritis
- ▶ Renal Manifestations of Metabolic Disorders in Children
- ▶ Renal Transplantation: Evaluation of Children and Donors
- ▶ Renal Tubular Acidosis

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Fabry Disease

34

Michael Beck

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Abstract

Fabry disease is a rare lysosomal storage disorder, caused by the deficiency of the enzyme alpha-galactosidase A that is responsible for the degradation of the sphingolipid globotriaosylceramide, also called Gb3. Due to the insufficient activity of alpha-galactosidase-A, Gb3 accumulates in a variety of cells of the body, resulting in progressive organ damage. Fabry disease is an X-linked disorder in which also females are affected, although often less severely than males. Clinically, this multisystemic disorder is characterized by neuropathic pain, gastrointestinal disturbances, typical skin lesions, sweating difficulties, and kidney and heart failure. Life expectancy is

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significantly reduced, whereby in both males and females, renal and cardiac diseases are the primary causes of death. In children, pain in hands and feet (acroparesthesia) and abdominal cramps are the leading symptoms; further clinical manifestations include corneal opacities, called cornea verticillata, and skin abnormalities (angiokeratoma). Albuminuria and an increased heart size represent the first signs of renal and cardiac manifestation that can be present already in young patients. Besides symptomatic and supplementary treatment such as analgesics, drugs for kidney dysfunction and for blood pressure control, enzyme replacement therapy is available for children with Fabry disease.

Keywords

Acroparesthesia · Alpha galactosidase A · Angiokeratoma · Cornea verticillata · Enzyme replacement therapy · Fabry disease · Globotriaosylceramide · Lysosomal storage disorder · Sphingolipid · X-linked disorder

Pathophysiology and Genetics

Fabry disease is an X-linked sphingolipid storage disorder caused by the deficiency of the lysosomal enzyme alpha-galactosidase A. Sphingolipids are complex lipids in which to the hydrophobic ceramide (composed of the long-chain sphingoid and a fatty acid) monosaccharides are bound. They are major components of cell membranes and have signaling and regulatory functions [1]. The sphingolipid globotriaosylceramide (Gb3) is composed of ceramide and the monosaccharides glucose and galactose; it is degraded within the lysosomes by the enzyme alpha-galactosidase A (Fig. 1). A genetic defect of this enzyme leads to the accumulation of Gb3 in various cell types such as endothelial cells, different kidney cells, and cardiac and nerve cells. The occurrence of the storage material in a great number of tissues and organs explains the multisystem character of Fabry disease. Aerts and colleagues have found that the level not only of Gb3, but also of the deacylated form, namely globotriaosylsphingosin (LysoGb3), is increased in several organs of Fabry patients and seems to play an important role in the pathogenesis of this lysosomal storage disorder as it inhibits alpha-galactosidase A activity and promotes proliferation of smooth muscle cells [2].

Alpha-galactosidase A is encoded by the *GLA* gene that was mapped to the long arm of the X-chromosome in the region Xq22.1 and contains seven exons. More than 960 pathogenic variants have been described in the *Human Gene Mutation Database (HGMD)*, whereby most of them (75%) are point mutations, and only a few (about 5%) encompass the splicing region [3].

The degree of X-chromosome inactivation (XCI) plays an important role in the phenotypic expression of females with Fabry disease: XCI is a mechanism that takes place in early female development by which one of the two X-chromosomes is inactivated in order to achieve gene dosage equivalence between females and males who carry a single X-chromosome. This process results in an average inactivation ratio of 50:50 with normal distribution. However, a percentage of females will have – without any obvious reason – unequal (skewed) XCI with a preferential inactivation of the same X-chromosome. Therefore, the disease severity and progression in Fabry females depends – at least in part – from the ratio of wild-type to mutated X-chromosome inactivation [4, 5].

Fabry disease is a rare condition, but probably the most frequent lysosomal storage disorder in humans. Formerly, the incidence has been estimated to range from 1 in 40,000 to 1 in 117,000 male live births [6]. A number of newborn screening studies have been performed in the last few years, however, and revealed that Fabry disease seems to be much more prevalent than it was anticipated from former observations. The prevalence, calculated from these studies, ranges from 1/1100 boys in Italy to 1/7057 (boys and girls) in Japan [7]. It has to be taken into account, however, that, in general, newborn screening detects more

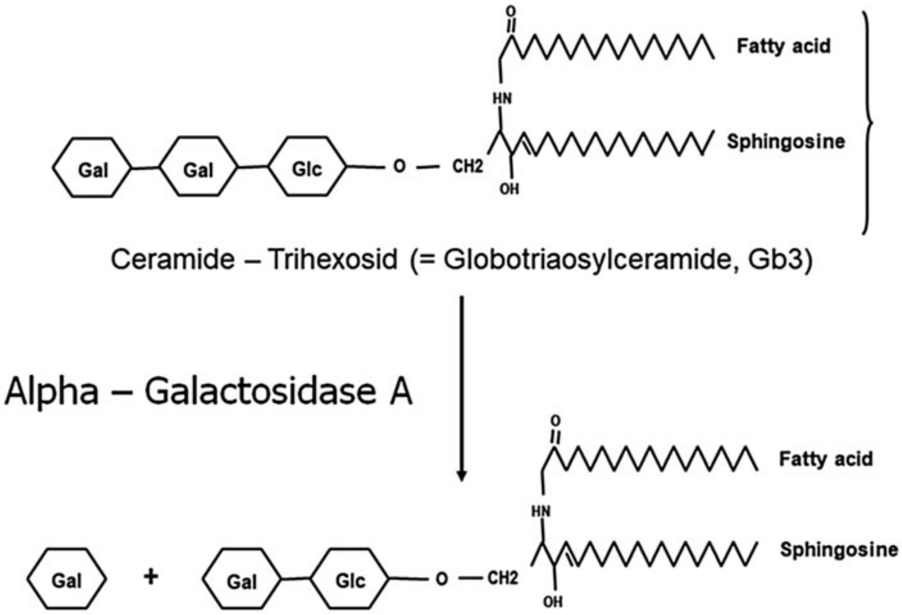

Fig. 1 Alpha galactosidase A splits galactose off the sphingolipid globotriaosylceramide (Gb3)

cases of a late-onset phenotype than those of a classic phenotype [8].

Clinical Manifestations

Neuropathic Pain and Gastrointestinal Symptoms

Peripheral neuropathy represents the first striking symptom in children with Fabry disease, which can be explained by Gb3-accumulation in vasa nervorum and small nerve axons, leading to degeneration of small unmyelinated fibers [9]. Boys and girls complain of pain, often associated with fever and reduced heat and exercise tolerance. Pain, in Fabry disease is also called acroparesthesia, described as burning, often associated with a deep aching sensation [10]. In boys, acroparesthesia can occur already at the age of 3 years [10].

Accumulation of Gb3 in autonomic small fibers explains other clinical manifestations of Fabry disease that are seen already in childhood: hypohydrosis, reduced heart rate variability, and gastrointestinal disturbances such as abdominal cramps, diarrhea, and nausea.

Angiokeratomas

The first characteristic skin abnormality (angiokeratomas) can be seen at the age of about 6 years. Angiokeratomas are vascular anomalies of the superficial vascular plexus that form dark red to black papules or nodules covered by epidermal hyperplasia (hyperkeratosis). In childhood, angiokeratomas can be very discrete, for example, surrounding the umbilicus, and in adults they are present at the buttocks, lower back, and genitalia (Fig. 2). Angiokeratomas can be seen also on limbs, hands and feet, and lips.

Eyes and Ears

A specific ophthalmological manifestation of Fabry disease is represented by the condition, cornea verticillata, which can be noted even in patients below 5 years of age. Cornea verticillata consists of

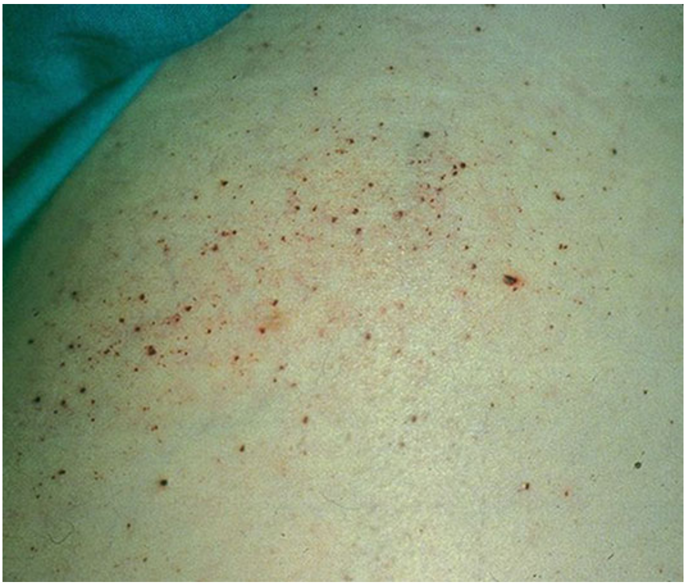

Fig. 2 Angiokeratomas at the back

whorl-like opacities, located in the superficial corneal layer. It usually does not lead to visual impairment. Other ocular changes include tortuosity of retinal vessels and lens cloudiness. Kalkum and colleagues demonstrated that children with eye findings were more severely affected than those without ocular manifestations [11].

Sensorineural and conductive hearing loss is rather common in Fabry disease and may be present in childhood. The involvement of the auditory system can be explained by hyperplastic mucosa and seropurulent effusions of the middle ear and Gb3 accumulation in vascular endothelial and various ganglion cells [12]. An analysis of audiograms of 47 pediatric patients with Fabry disease revealed that affected boys and girls showed increased hearing thresholds at all frequencies, compared to healthy children, in particular at ultrahigh frequency. Five patients had sensorineural hearing loss [13]. Tinnitus and vertigo can be also observed in children with Fabry disease [10].

Kidney

Renal involvement represents the main cause of morbidity and mortality in Fabry disease. Clinical features of Fabry nephropathy have been well known for many years. Proteinuria develops as the first clinical sign in childhood and adolescence, followed by continued decline of kidney function and often reaching end-stage kidney disease (ESKD) in the fourth or fifth decade of life [14].

Analysis of renal biopsies from children reveals storage material in all endothelial cells, in glomerular mesangial cells and podocytes, in distal tubular cells and vascular smooth muscle cells (Fig. 3a, b). It is remarkable that Gb3 accumulation in podocytes is detectable in patients with normal glomerular filtration rate (GFR) and before the development of pathological albuminuria [15]. Histological investigation demonstrates Gb3 accumulation even in the fetal kidney tissue at 19 weeks' gestation, many years before deterioration of kidney function can be found [16].

Heart

The cardiac manifestations of Fabry disease also begin in childhood. Echocardiographic evaluation, performed in 20 children and adolescents under the age of 18 years, revealed that all patients had a left ventricular mass, indexed to height ($LVM/h^{2.7}$), above the 75th percentile of that of healthy controls; yet, blood pressure was in the

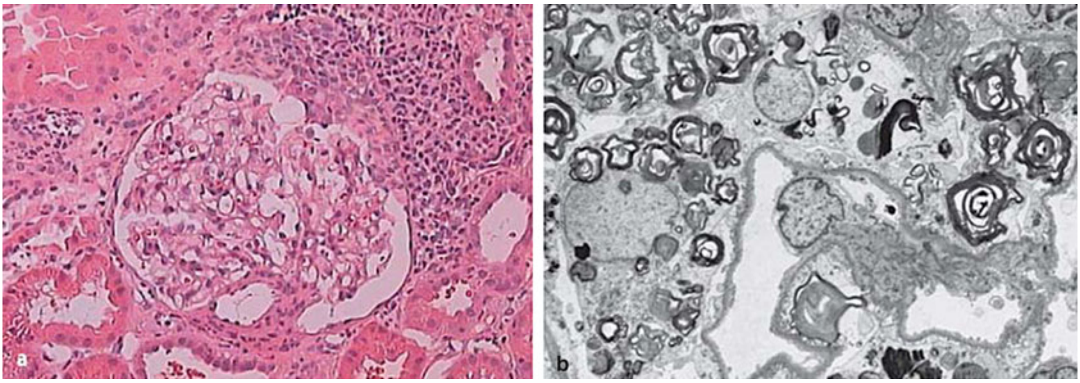

Fig. 3 Kidney biopsy of a Fabry patient (From: [31], with permission). (**a**) The foamy material within glomerular structure represents deposition of Gb3 inclusions that were removed by the embedding process. (**b**) Electron microscopy picture: Osmiophilic zebra bodies in podocytes and glomerular endothelial cells

normal range [17]. Two-hour Holter electrocardiography revealed significantly reduced heart rate variability in males, but not in females, indicating a reduction in parasympathetic stimulation of the heart. Further clinical features that may appear already in young patients include arrhythmias, chest pain, and valvular disease, including mitral valve prolapse [10].

Central Nervous System (CNS)

Stroke and transient ischemic attacks (TIAs) are very common in adult males and females with Fabry disease and are probably caused by a combination of endothelial dysfunction and abnormal function control of the vessels. Whereas cerebrovascular events seem to be very rare in childhood, other symptoms of CNS manifestation such as poor cognitive and executive functioning are apparently more common [18].

Diagnosis

The diagnosis of Fabry disease is made rarely in children, because the first symptoms such as pain and gastrointestinal complaints are rather unspecific, and not every pediatrician is familiar with this rare storage disorder. Therefore, in families with at least one relative with Fabry disease, systematic screening of children must be performed. In boys, the diagnosis can be easily confirmed by measurement of alpha-galactosidase A preferably in leukocytes, but also in plasma or a dried blood spot. In order to confirm the diagnosis in girls, analysis of the *GLA* mutation, which is already known in the affected family members, is necessary, as in affected females the enzyme activity, measured in leukocytes, is often normal.

Due to the lack of specific symptoms, it is often very difficult to make the clinical diagnosis in children of families in which no further Fabry case is known. The combination of burning pain in hands and feet together with sweating difficulties and gastrointestinal symptoms should raise the suspicion of Fabry disease. Also, angiokeratomas may provide an indication of Fabry disease, although it has to be taken into consideration that this skin abnormality is not specific as it can be observed also in other lysosomal storage disorders such as fucosidosis or sialidosis, as well as in apparently healthy individuals [19].

Many patients experience a previous misdiagnosis such as rheumatological disease, arthritis, erythromelalgia, or a neuropsychological disease. Whereas in boys the suspicion of Fabry disease can be confirmed rapidly by enzyme assay, in girls the diagnosis can be more difficult: As noted above, normal enzyme activity does not exclude Fabry disease; therefore, mutational analysis is necessary. If, however, the molecular study reveals a variation of unknown significance, additional clinical and biochemical investigations become necessary. A slit-lamp examination should be performed in order to check for the presence of cornea verticillata, which can be found in about two-thirds of girls [10].

In the past, increased plasma or urinary concentrations of Gb3 were used as a biomarker to monitor progression of Fabry disease. In several studies, however, it has been found that Gb3 concentrations are a poor reflection of the degree of clinical manifestations and therapeutic outcome. Presently, LysoGb3 concentrations are used for the diagnosis and treatment monitoring in Fabry disease. As serum LysoGb3 is significantly elevated not only in males, but also in affected females, it represents a better biochemical diagnostic marker for the identification of Fabry heterozygotes [20]. If the diagnosis of Fabry disease is made in an individual, genetic counseling including pedigree analysis of the whole family is mandatory.

Treatment

General Remarks

Fabry disease is a multisystemic disorder that requires comprehensive and multifaceted management. The establishment of a multidisciplinary team is recommended and should include a pediatric nephrologist, pediatric cardiologist, pediatric neurologist, and other specialists such as a gastroenterologist or psychologist. The coordinator of the team may be a pediatrician or geneticist. Examinations and treatment of the children should be preferably performed in a center that has experience in the management of Fabry disease.

Pain and Gastrointestinal Symptoms

Recommendations regarding the usage of different drugs for the treatment of Fabry-related pain in children are derived from the experience of adult patients with polyneuropathy, trigeminal neuralgia, and other neuropathic conditions. Pain medication should be started at low dose and escalated slowly to therapeutic or adverse effect. Anticonvulsants are commonly used for treatment of Fabry-related pain. Carbamazepine, alone or in combination with pregabalin or gabapentin, can be the first choice of treatment in Fabry disease. One should be aware, however, that carbamazepine bears the risk of exacerbation of autonomic dysfunction. Antidepressants such as venlafaxine or duloxetine are also feasible alternatives. Tricyclic antidepressants should be used with caution because of their anticholinergic effects and the potential to worsen autonomic symptoms [21].

Gastrointestinal problems such as nausea, vomiting, and diarrhea, which are common and important early symptoms in Fabry disease, respond very well to enzyme replacement therapy [22]. Additionally, dietary restrictions and eating small frequent meals are recommended.

Skin

Laser therapy is a consideration for the treatment of angiokeratomas. The results of various laser methods, however, have been mixed or discouraging, and laser therapy may lead to scars and does not prevent the formation of new lesions [23].

Eyes and Ears

The corneal opacity (cornea verticillata) is of diagnostic value but does not impair vision; therefore, any treatment is not required. Audiological investigation should be performed yearly, for the management of hearing loss, and hearing aids or even cochlear implants should be applied.

Kidney

A comprehensive evaluation of kidney function is needed that should include measured GFR (mGFR) and estimated GFR (eGFR) in all children with Fabry disease. As at early stages of progressive renal disease eGFR formulae using serum creatinine are relatively inaccurate, a more precise mGFR (iohexol or radionuclide tracers) should be preferred for assessment of kidney function [24]. However, as these methods may be technically difficult in very young children and/or may not be available in every hospital, eGFR may be used for regular assessment of kidney function at diagnosis and thereafter every 6 or 12 months. It is recommended to use at the first visit also mGFR, in addition eGFR, to determine whether the formulae for eGFR are accurate estimates for that individual patient. It is important also to measure urinary albumin and protein excretion at each visit, as the presence of pathological albuminuria or proteinuria is sufficient to indicate the diagnosis of chronic kidney disease, even in the presence of normal GFR. However, it is clear that normal GFR and urinary protein and albumin levels do not exclude a progressive kidney injury [15]. Although a renal biopsy can show early pathological changes of the kidney, it has not been proven that initiation of treatment based on histopathological findings can improve clinical outcomes. However, a kidney biopsy is mandatory if a decline in kidney function or a substantial increase of protein excretion raises the suspicion of the presence of a concomitant disease, for example, an IgA- or IgM-nephropathy [25, 26].

For the treatment of pathological proteinuria, angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) can be used.

Heart

As mentioned in section “Heart,” cardiac complications such as left ventricular hypertrophy, arrhythmias, and valvular disease can occur in children with Fabry disease. Echocardiography and ECG should be performed at each clinical visit. The Holter test is necessary only if an abnormal rhythm is suspected or palpitations are reported. In order to prevent cardiovascular disease in later life, implementation of healthy lifestyle measures is recommended and should include management of blood pressure and weight control. Other medications, for example, calcium channel antagonists or statins in case of increased lipid levels, and therapeutic procedures such as pacemaker implantation, are in general necessary only in adult patients.

CNS

As the CNS is hardly involved in children with Fabry disease, an MRI should only be performed if a child experiences any neurological change that could be related to a cerebrovascular event. However, as cognitive deficits and depression are not uncommon in young Fabry patients, some children may need psychological support.

Enzyme Replacement Therapy

Enzyme replacement therapy (ERT) seeks to enhance enzyme function in lysosomes by regular intravenous infusions of a genetically engineered recombinant enzyme. In Europe and many other countries, two enzyme preparations are licensed, agalsidase alfa (Replagal®, Shire Takeda), produced in a human cell line, and algalsidase beta (Fabrazyme®, Sanofi Genzyme), produced in a Chinese Hamster Ovary (CHO) cell line. In the USA, only agalsidase beta has received marketing authorization. Although both enzyme preparations are biochemically and structurally very similar, there is a considerable difference in the dosage. The recommended dosage for agalsidase alfa is 0.2 mg/kg every other week (EOW), and for agalsidase beta 1.0 mg/kg EOW. Several studies have demonstrated that treatment with both enzymes can improve or at least delay some of the clinical manifestations such as the pain and kidney and heart dysfunction [27].

Agalsidase alfa and beta are safe and well tolerated in adults and children. Children receiving ERT experience a reduction of neuropathic pain and gastrointestinal symptoms, resulting in an improvement of exercise performance and of quality of life [28]. Kidney biopsies, performed in young patients several years after initiation of ERT, showed clearance of glomerular endothelial and mesangial cell inclusions, and in four patients, who received the highest dose of agalsidase, a substantial clearance of podocyte inclusions could be detected. Albuminuria normalized in some patients [29].

The decision when to start ERT denotes a difficult decision as it has important implications not only for the child, but also for the whole family. A negative impact on social activities, the need for repeated venous access, and the potential for infusion reactions should be taken into consideration. However, these factors should be balanced against the chance of clinical benefits that can be gained from preventing irreversible organ damage or at least reducing disease progression.

There is a general consensus that ERT should be initiated in children as soon they show any major organ involvement, regardless of age or sex. Signs and symptoms that warrant treatment with an enzyme preparation are neuropathic pain, gastrointestinal disturbances, decline in kidney function, pathological albuminuria or proteinuria, and signs of heart involvement (cardiomyopathy or arrhythmia). Whether asymptomatic boys around age 8–10 years should receive ERT, based on the evidence of storage material in a kidney biopsy, prior to the onset of albuminuria or other clinically significant renal findings, remains a matter of debate [15]. Asymptomatic girls with Fabry mutations should be followed closely according to the same schedule of clinical examinations as male patients. They should be treated, however, as soon as they show any clinical manifestation.

Chaperone Therapy

An efficient mechanism exists that controls exact folding of newly synthesized enzymes in each cell. Some missense mutations that do not affect the catalytic site, but the accurate folding of lysosomal enzymes, prevent them from reaching the lysosome, so that they cannot fulfil their function. However, some mutant enzymes might regain at least partial catalytic activity if they could acquire their correct confirmation. The amino sugar migalastat (Galafold®, Amicus Therapeutics) is a competitive inhibitor of the enzyme alpha-galactosidase A that, at subinhibitory concentrations, functions as a so-called chemical chaperone leading to correct confirmation of the mutant enzyme and thereby increasing its catalytic activity. Migalastat is approved for patients 16 years of age and older with an amenable *GLA* variant, which means a mutation that affects not the catalytic site, but the folding of the enzyme [30].

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Abstract

Three distinct types of primary hyperoxaluria (PH) have been identified based on molecular genetics and biochemical analyses. All types are characterized by loss of function of key enzymes of the hepatic glyoxylate metabolism. The prognosis of the disease is dependent on the type of PH. PH1 is the most common and the most severe form. Patients with PH1 have a high risk of progressing to end-stage kidney disease (ESKD) and of developing systemic manifestations related to oxalate depositions in tissues (systemic oxalosis). In comparison, PH2 carries an intermediate risk of kidney failure, usually in adulthood, and a lower risk of systemic oxalosis. PH3 is the most favorable form; ESKD has only been reported anecdotally in PH3, and, remarkably, symptomatic adults are rarely diagnosed.

Measurement of urinary oxalate and genetic testing allow screening and rapid diagnosis of PH. Precise genotyping has important therapeutic implications. RNA interference treatment represents one of the first examples of precision medicine in nephrology. Nonetheless, hyperhydration and vitamin B6 (pyridoxine) supplementation in susceptible PH1 genotypes continue to represent the mainstay of treatment for many patients. However, effective and affordable treatment for PH remain an unmet need in many areas of the world.

In addition to PH, hyperoxaluria can be secondary to intestinal malabsorption, to ingestion of oxalate precursors, or to specific dietary conditions. Without appropriate measures for prevention and treatment, secondary hyperoxaluria can cause severe kidney function impairment that may progress in some cases to ESKD.

Keywords

Hyperoxaluria · Primary hyperoxaluria · Secondary hyperoxaluria · Glyoxylate metabolism · Interferent RNA therapy · Vitamine B6 · Combined liver-kidney transplantation

Introduction

Primary hyperoxalurias (PH) are a group of autosomal recessive disorders of glyoxylate metabolism with variable incidence in different regions of the world. This chapter discusses the major biochemical, genetic, and therapeutic advances in these diseases.

The enzymatic defects cause overproduction of oxalate, primarily by the liver, which increases the oxalate load to the kidneys. Oxalate is a highly insoluble end-product of collagen metabolism in humans. The first symptoms are related to hyperoxaluria (i.e., urolithiasis and/or nephrocalcinosis), which progressively impair the glomerular filtration rate (GFR). A second phase of tissue damage develops when the kidneys become unable to excrete the entire oxalate load, causing oxalate depositions in all tissues. This phase is termed systemic oxalosis.

The diagnosis of PH relies on measuring urine oxalate (Uox) excretion and performing genetic tests. This allows classifying most cases of PH into three forms, namely, type 1, 2, and 3 PH. PH1 is the most common and severe form. Rarely, the phenotype remains uncertain and does not correspond to a known PH subtype from the genetic standpoint.

Current management includes conservative measures, which for many years were followed by combined liver-kidney transplantation in PH1. New therapeutic approaches targeting the glyoxylate pathway in the liver have recently been developed and are likely to delay in the future chronic kidney disease (CKD) progression in all types of PH and to replace liver transplantation in PH1.

Secondary hyperoxaluria may occur in conditions characterized by excess of oxalate precursors or increased intestinal oxalate reabsorption (enteric hyperoxaluria), particularly in malabsorption syndromes. Excess dietary intake has also been reported to cause secondary hyperoxaluria. Secondary hyperoxaluria must be excluded in all patients investigated for PH.

Definition

Oxalate is the dianion of oxalic acid (HOOC-COOH), an organic acid with a molar mass of 90 g.mol^{-1} in its anhydrous form. Measurement of Uox excretion in a timed-urine collection (preferably 24 h) is the gold standard to diagnose hyperoxaluria (Table 1). In infants and young children, spot urine samples may be used to measure the Uox:Ucr (urinary creatinine) ratio. The Uox:Ucr ratio is a reliable screening method, but results must be interpreted according to age (Table 1). Uox can be measured with enzymatic methods, using commercially available kits, liquid chromatography (LC), and gas chromatography after derivatization (GC). Enzymatic methods have the advantage of being easily automatized. Chromatographic methods are more laborious but allow simultaneous assessment of oxalate and citrate (LC) or oxalate and glycolate (GC). In all types of PH, the level of Uox excretion tends to correlate with kidney function outcome [3].

In PH, the Uox excretion is usually ≥1 mmol/1.73 m^2/24h, with the normal range being <0.5 mmol/1.73 m^2/24 h (Table 1).

Table 1 Reference values for urinary oxalates excretion [1, 2]

Uox:Ucr ratio	
<2 months	<0.28 (mmol/mmol)
2 months–2 years	<0.14 (mmol/mmol)
2 years–12 years	<0.10 (mmol/mmol)
>12 years	<0.08 (mmol/mmol)
24 h Uox excretion	
All ages and sex	<0.5 mmol/1.73 m^2/24 h
Adult male	0.08–0.49 mmol/24 h
Adult female	0.04–0.32 mmol/24 h

Uox, urinary oxalate

Primary Hyperoxalurias

Primary hyperoxalurias are rare inherited disorders of glyoxylate metabolism characterized by increased excretion of Uox leading to recurrent renal stone formation, nephrocalcinosis, and ultimately kidney function impairment. When advanced kidney failure develops, deposition of nonsoluble oxalate in tissues rapidly occurs, causing systemic oxalosis [1].

Three distinct genetic types of PH have been described, namely, PH1, PH2, and PH3. The overall prevalence is 1 per 58,000, based on population analysis approaches [4]. Approximately 70% of patients have PH1, 10% have PH2, and 10% have PH3. In the remaining 10% of patients, a genetic cause cannot be identified. The relative proportion of the different types of PH vary slightly in different regions of the world.

Metabolic Defects

PHs are rare disorders of the glyoxylate metabolism in the liver, causing excessive endogenous production of oxalate. PH per se is not a kidney disease, but since the kidneys are responsible for oxalate clearance, PHs manifest predominantly as a kidney and urinary tract disease, with recurrent nephrolithiasis and/or nephrocalcinosis.

All subtypes of PH are inherited in an autosomal recessive mode and are secondary to the loss of function of the mutated enzyme. Figure 1 summarizes the main metabolic pathways involved in PHs.

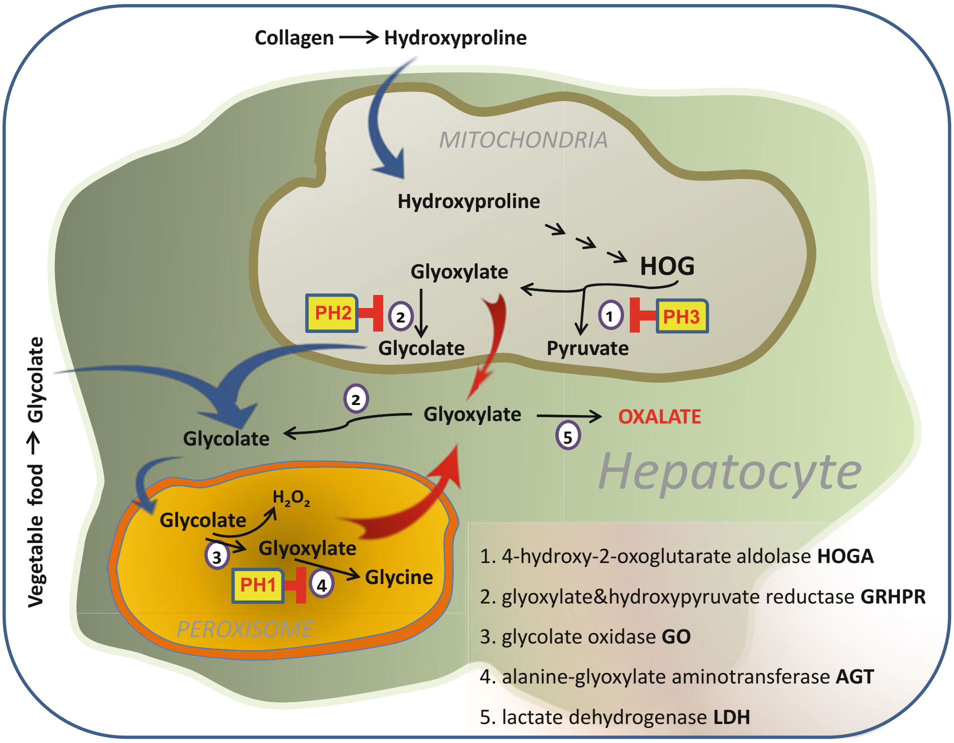
Oxalic acid is found in virtually all dietary products in quantities that depend on the source and processing of the product. The oxalate content of a balanced diet does not represent a health issue in the absence of conditions that cause secondary hyperoxaluria.

It is always important to differentiate PHs, which are rare conditions that do not respond well to dietary restrictions, from secondary hyperoxalurias, which are more frequent and potentially treatable with dietary adjustments. Sometimes, this can be challenging in the absence of genetic investigations.

Despite the ability of kidneys to filter efficiently high amounts of small compounds such as oxalate, poor solubility of oxalate in water and high concentration of other solutes in renal tubules limit the solubility of calcium oxalate (CaOx), causing precipitation of insoluble CaOx crystals in patients with PHs. Progressively, interstitial tissue deposition of CaOx occurs, causing severe inflammatory responses that eventually lead to fibrosis and ESKD.

Genetic Analysis

A clinical and biochemical suspicion of PH always requires genetic confirmation, which can be performed using targeted sequencing (Sanger)


Collagen → Hydroxyproline
MITOCHONDRIA
Hydroxyproline
Glyoxylate
HOG
PH2
2
Glycolate
1
PH3
Pyruvate
2
Glyoxylate
OXALATE
5
Glycolate
Hepatocyte
Vegetable food → Glycolate
Glycolate
H₂O₂
3
Glyoxylate
PH1
4
Glycine
PEROXISOME

1. 4-hydroxy-2-oxoglutarate aldolase **HOGA**
2. glyoxylate&hydroxypyruvate reductase **GRHPR**
3. glycolate oxidase **GO**
4. alanine-glyoxylate aminotransferase **AGT**
5. lactate dehydrogenase **LDH**

Fig. 1 Metabolic pathways in primary hyperoxalurias. (Courtesy Dr R Belostotsky, Jerusalem, Israel)

or next-generation sequencing (NGS, gene panels/whole exome) of the three causative genes: *AGXT* (PH1), *GRHPR* (PH2), and *HOGA1* (PH3). Since there is only modest genetic heterogeneity in PH and all involved genes are small, targeted Sanger sequencing remains a fast and cost-effective approach.

Defining the type of PH has important prognostic and therapeutic consequences. It defines the possibility of treatment with RNA interference. Some missense mutations (e.g., p.Gly170Arg) of the *AGXT* gene are more likely to respond to conservative treatment with hyperhydration and pyridoxine and are associated with better long-term kidney survival [4–10]. To date, no clear genotype-phenotype correlations has been established in PH2 and PH3 [11, 12]. Genotype information is likely to become increasingly important in future precision medicine approaches.

The prevalence of copy number variations (CNVs) in PH has not been systematically analyzed but appears to be low (<1%; personal communication, B Beck). In rare cases where genetic sequencing identifies only one heterozygous pathogenic variant in patients with a high clinical suspicion of PH, NGS results should be checked for CNVs. Since most heterozygous CNVs would be missed by Sanger sequencing, molecular diagnostics should be complemented by multiplex ligation-dependent probe amplification assay (MLPA) in these patients. MLPA probes are commercially available for *AGXT* and *GRHPR,* but not for *HOGA1* [13].

Alternatively, the expression and enzyme activity of the *AGT, GRHPR,* and *HOGA1* gene products can be tested in tissues obtained from a liver biopsy. This classical methodology has been largely abandoned in favor of noninvasive genetic testing.

Prenatal diagnosis cannot be performed with fetal ultrasonography or by measuring amniotic fluid oxalate concentration. It can only be performed with genetic analysis using noninvasive techniques or DNA specimens obtained from chorionic villi or amniocytes. Prenatal diagnosis can therefore be proposed only to families with index cases that have been genetically characterized. It allows identification of affected and non-affected fetuses.

Primary Hyperoxaluria Type 1

Pathophysiology

PH1 (OMIM 259900) is an autosomal recessive disorder caused by functional defects of the alanine-glyoxylate aminotransferase (AGT) peroxisomal enzyme, leading to oxalate overproduction (Fig. 1). AGT is a liver-specific enzyme that is pyridoxal-5′-phosphate-dependent. Symptoms develop if the AGT activity is insufficient or if AGT is mistargeted to the mitochondria, which may explain enzymatic heterogeneity. The kidneys are the initial target of oxalate overproduction. The first symptoms are related to hyperoxaluria (i.e., urolithiasis and/or nephrocalcinosis), which progressively compromises the integrity of the renal parenchyma, causing CKD and ultimately ESKD. Renal damage is multifactorial, including oxalate-mediated tubular damage, nephrocalcinosis with intratubular and interstitial calcium oxalate (CaOx) depositions, interstitial inflammation and fibrosis, obstruction by stones, and often superimposed infection [1]. A second phase of tissue damage in PH1 occurs when the decline in GFR reaches stage 3 CKD (i.e., 30–59 mL/min per 1.73 m^2). In this condition, the kidneys become unable to excrete the entire oxalate load, causing a rise in plasma oxalate concentration (normal Pox levels: <6 to 17 μmol/L) that exceeds the plasma saturation threshold (around 30 to 40 μmol/L). A multisystem injury secondary to CaOx depositions in virtually all tissues develops; this condition is referred to as systemic oxalosis [14–17]. Bone deposition can be massive, resulting in debilitating pain and fractures (Fig. 2). After kidney transplantation, the mobilization of oxalate from stores can be extremely important, in particular from bones, and may last for months and even years [18]. This can cause deposition of oxalate in the newly transplanted kidney and possibly graft failure, a well-known complication in undiagnosed PH1 patients receiving isolated kidney transplantation [18].

Severe systemic oxalosis is primarily observed in PH1; it is rarely reported in patients with ESKD secondary to other types of PH with moderately elevated Pox levels [15, 19]. Interestingly, the substantial contribution of elevated Pox levels to

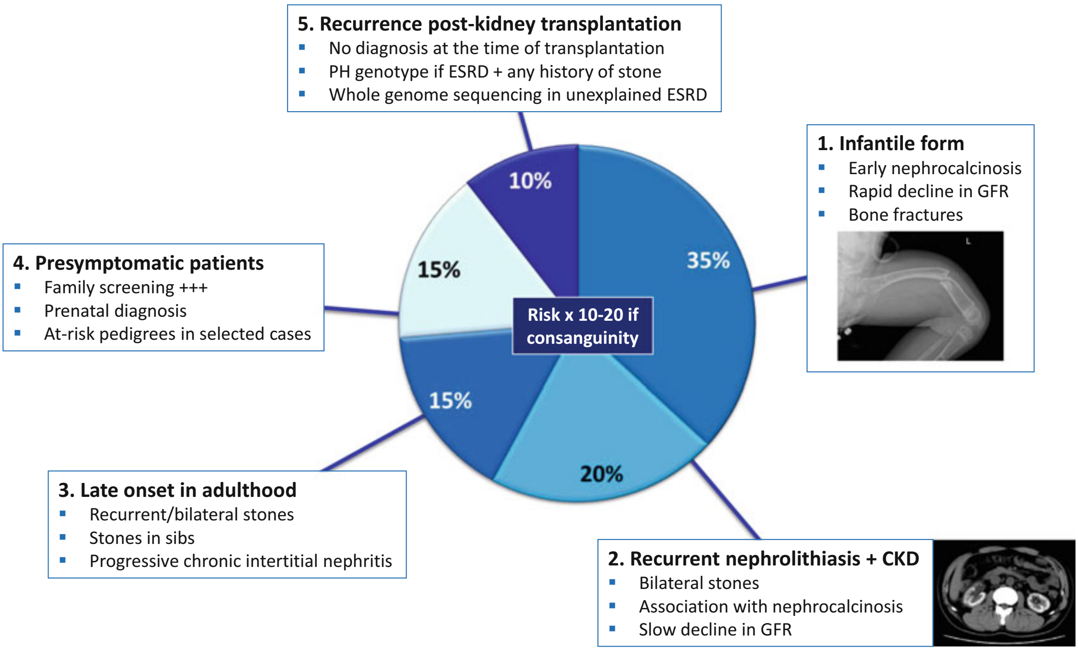

5. Recurrence post-kidney transplantation
No diagnosis at the time of transplantation
PH genotype if ESRD + any history of stone
Whole genome sequencing in unexplained ESRD
1. Infantile form
Early nephrocalcinosis
Rapid decline in GFR
Bone fractures
L
4. Presymptomatic patients
Family screening +++
Prenatal diagnosis
At-risk pedigrees in selected cases
10%
15%
35%
Risk x 10-20 if consanguinity
15%
20%
3. Late onset in adulthood
Recurrent/bilateral stones
Stones in sibs
Progressive chronic intertitial nephritis
2. Recurrent nephrolithiasis + CKD
Bilateral stones
Association with nephrocalcinosis
Slow decline in GFR

Fig. 2 Presentation of PH1: a heterogenous unpredictable disease [1]

the pro-inflammatory state and increased cardiovascular risk of the latter population have not received a lot of attention.

Epidemiology

PH1 is the most common form of PH worldwide. Its clinical incidence in Western Europe approximates 1:100,000 live births per year. PH1 accounts for 1% to 2% of pediatric patients with ESKD, according to registries from Europe, the USA, and Japan. It is significantly more frequent in North African and Middle East nations, where consanguineous marriages are common. In these countries, PH1 is responsible for up to 10% of pediatric ESKD cases and sometimes even more [17, 20, 21]. Twenty to thirty percent of all patients with PH1 present with ESKD at diagnosis [3, 22]. The infantile form often presents as a life-threatening condition. This form is particularly severe because of the combined effects of a very high urinary oxalate load and the immaturity of the kidney function in this age group. Half of these infants have ESKD at the time of diagnosis, and 80% progress to ESKD by the age of 3 years [23, 24]. Unfortunately, the diagnosis is often made too late in most patients with PH, after complications of oxalosis have developed.

Diagnosis and Outcomes

Diagnosis

PH1 should be considered in any child with urolithiasis/nephrocalcinosis and in adults with recurrent CaOx stone events.

The diagnosis is first suspected based on clinical symptoms (Fig. 2). *Patients may present with different clinical pictures*:

- Infants and even neonates with advanced renal failure and nephrocalcinosis. These patients often present with failure to thrive, acute pyelonephritis, vomiting, pallor due to anemia, bone fractures, and other symptoms related to uremia.
- Nephrolithiasis at any age, usually recurrent and bilateral, often associated with decreased GFR.
- When patients reach CKD stage 3, severe extrarenal manifestations may develop [1]:
  - The main site of deposition of oxalate is the skeleton (Fig. 2), leading to pathologic

  fractures, bone deformations, severe bone pains, and a specific oxalate osteopathy on X-ray [25].
  - All organs can be involved: myocardium, retina, joints, vessels, skin, marrow, nerves, liver, brain, thyroid, gonads, etc. [1, 26–30].

The main biomarkers for diagnosis are in the urine:

- Microscopic urine crystal examination and infrared spectrometry show monohydrated calcium oxalate (whewellite).
- Urine oxalate >0.5 mmol/1.73 m^2 BSA per 24-h or Uox:Ucr >0.10 mmol/mmol.
- Urine glycolate >0.5 mmol/1.73 m^2 BSA per 24 h.

Plasma oxalate concentration cannot be regarded as a diagnosis marker, since it is influenced by GFR [31]. The upper limit of the normal range varies between 6 and 17 μmol/L, depending on the methodology [16]. Plasma oxalate measurements are mostly helpful in the follow-up of dialyzed and transplanted patients.

Genotyping is required to confirm the diagnosis of PH1 and to guide treatment.

AGT enzyme activity can be assessed on liver biopsy specimens, but this invasive approach has been replaced by genotyping, which also provides reliable genotype/phenotype correlations.

Outcome

Patients with PH1 often experience acute deterioration of their GFR that may be triggered by urological issues, including acute pyelonephritis, ureteral obstruction, open urological surgery, or extracorporeal lithotripsy, or by non-urological conditions, such as acute dehydration, acute kidney injury of any type of surgery or general anesthesia. PH1 is a devastating disease that leads to ESKD within the first two to three decades of life in most cases.

Treatment: Supportive Measures

Limited supportive measures are available for patients with PH1. They are more efficient if started early, even before confirmation of the diagnosis by genetic testing. Symptomatic treatment relies on continuous hydration over the entire 24 h period, inhibition of CaOx crystallization, and pyridoxine therapy in responsive patients (Table 2) [32–34].

Restriction of dietary oxalate intake (spinach, rhubarb, cocoa, tea, asparagus, star fruit, Swiss chard, etc.) has limited impact because dietary oxalates contribute only marginally to the renal oxalate load in PH. Dietary calcium should not be restricted because calcium binds oxalate in the intestine to form insoluble CaOx complexes that are eliminated in the stools [33]. Ascorbic acid supplementation should be avoided because ascorbic acid is a precursor of oxalate [34, 35].

Dialysis

In principle, dialysis should always be avoided in PH. Nonetheless, dialysis is necessary in patients presenting with ESKD or in those that cannot be treated with transplantation before they need renal replacement therapy [33]. Oxalate is a small molecule, easily cleared by hemodialysis membranes. However, conventional hemodialysis is unable to match the hepatic production (4 to 8 mmol per day in adults); therefore, large amounts of oxalate are stored in the body when patients are treated with dialysis. Intensive dialysis strategies, including daily hemodialysis or combined hemo- and peritoneal dialysis, can remove more oxalate, but the balance remains positive and predialysis Pox levels usually are greater than 100 μmol/L. Theoretically, multiple short hemodialysis sessions per day could balance the oxalate overproduction by the liver, but these are not acceptable in terms of quality of life.

Transplantation

Kidney transplantation restores the kidney function, but only liver transplantation corrects the metabolic defect, preventing recurrence of the disease in the transplanted kidney. Therefore, most patients require a dual liver-kidney transplantation, either simultaneous or sequential (liver first and then kidney), according to the burden of oxalates and to local access to organs [36, 37]. In principle, organ transplantation in PH should always be preemptive to reduce the mortality and the morbidity caused by systemic

Table 2 Supportive measures in patients with PH1 and preserved GFR [33, 34]

Treatment	Recommendation	Goal of therapy
Hydration	**Water, 3 L/m^2/24h** *Tube feeding/gastrostomy in children*	**Uox <0.4 mmol/L and** **Uca <4 mmol/L**
Crystallization inhibitor	**Citrate, 0.10 to 0.15 g/kg/24 h** *Potassium citrate as long as GFR is preserved, then sodium citrate*	**Decreased number of stone episodes** **Increased urine citrate** **Decreased urine crystal volume**
Pyridoxine (AGT cofactor)	**Vitamin B6 (pyridoxal-phosphate), 5–20 mg/kg/day** *Start with 5 mg/kg/day, then increase the dose by steps up to a maximal dose of 20 mg/kg/day*	**Uox reduction >30%** **Useful only in selected *AGTX* mutations: p. Gly170Arg/p.Phe152Ile**
Reducing intestinal oxalate reabsorption	Oxalate intake restriction *Oxalobacter formigenes*	Limited long-term reduction of Uox and Pox *(dietary oxalates make a limited contribution to the total oxalate pool in PH)*

GFR, glomerular filtration rate; Ox, oxalate; Uox, urinary oxalate; Uca, urinary calcium

oxalosis. With the new therapeutic approaches (see below), kidney transplantation will probably be postponed in most patients with CKD, and liver transplantation may be restricted to those patients that cannot have access to these treatments.

Treatment: RNA Interfering Agents

RNA interference (RNAi) is a naturally occurring mechanism for regulating gene expression. Small interfering RNAs (siRNAs) allow selective depletion of mRNA molecules that encode for target proteins. In recent years, this technology has been used to develop innovative therapies for PHs.

The first identified target for siRNA therapy was glycolate oxidase (GO). This enzyme is exclusively expressed in hepatocytes and is believed to play a role only in glyoxylate metabolism. GO catalyzes the oxidation of glycolate to glyoxylate, which is the substrate for oxalate synthesis (Fig. 1). By decreasing glyoxylate production, inhibition of GO in PH1 decreases urinary oxalate excretion. The safety of GO suppression in humans was supported by the incidental discovery of GO deficiency in an 8-year-old boy with asymptomatic glycolic aciduria, associated with normal kidney and hepatic functions [38]. Lumasiran, an investigational GO-targeting siRNA, has been recently developed. Targeted delivery of siRNAs to the liver was obtained by conjugation to N-acetylgalactosamine (GalNAc). This allows direct uptake of the drug by asialoglycoprotein receptors, which are exclusively expressed in hepatocytes. The 6-month double-blind phase of a randomized, placebo-controlled phase 3 study evaluating lumasiran in 39 patients with PH1 has recently been completed [39]. The study included children aged more than 6 years and adults with genetically proven PH1 and eGFR >30 mL/min per 1.73 m^2. Lumasiran was administered subcutaneously at 3 mg/kg monthly for 3 months, followed by quarterly doses. The least squares mean change from baseline in 24 h Uox excretion was −65.4% in the lumasiran group and −11.8% in the placebo group. The proportion of lumasiran-treated patients who achieved normalization or near normalization of 24 h Uox excretion at 6 months was 84%, compared to 0% in placebo-treated patients. There were no serious or severe adverse events. The most common adverse event was a mild and transient injection site reaction in 38% of lumasiran-treated patients. Lumasiran has been recently approved by several health technology assessment bodies worldwide.

In addition, nedosiran, a siRNA targeting the lactate dehydrogenase A (LDHA) enzyme, has been recently developed. Similarly to lumasiran, conjugation with GalNAc allows selective silencing of LDHA in the liver, preventing off-target effects in other tissues, especially in muscles. Since LDHA mediates the final enzymatic reaction of oxalate production (Fig. 1), nedosiran can be beneficial in all types of PH. In a phase 1 study including 15 patients with PH1 and 3 patients with

PH2, nedosiran was shown to reduce Uox by 55% from baseline after 57 days, with 6 patients reaching normal 24 h Uox excretion rates [40]. Safety was excellent, with only injection site reactions reported in 5 of the 18 patients.

Treatment: Future Developments

High morbidity and mortality of liver transplantation and the excessive costs of siRNA-based targeted therapies will be the main drivers for the development of future therapeutic options [41]. The range of research and development include gene therapy that allows permanent correction of enzyme deficiencies, identification of pharmacochaperones for amenable (mistargeting) missense mutations, therapeutic targeting of genetic and/or environmental modifiers that have been identified in discordant families and in asymptomatic individuals with biallelic *HOGA1* mutations, and oxalate degrading therapies [42–58]. Development of biomarkers that help identify individuals at increased risk of renal injury and/or systemic oxalosis may allow the development of precision medicine approaches, including multimodal treatment strategies.

Stiripentol, an LDHA-targeted commercially available oral medication for Dravet syndrome, was recently reported to reduce significantly Uox in epileptic patients without PH1 and in one PH1 patient with preserved kidney function [59, 60]. The benefits of this treatment in PH are currently under investigation in a phase 2 clinical trial.

Primary Hyperoxaluria Type 2

Pathophysiology

PH2 (OMIM260000) is caused by complete or nearly complete loss-of-function of a ubiquitously expressed cytosolic/mitochondrial enzyme with glyoxylate/hydroxypyruvate reductase (GR/HPR, EC 1.1.1.26) activity [61]. Analysis of tissue samples from patients with PH2 has shown that the GR/HPR enzyme activity is highest in the liver but is also present in leukocytes and in the kidney [62]. Lack of GR/HPR leads to the accumulation of glyoxylate and hydroxypyruvate, which are metabolized by LDHA to oxalate and L-glycerate, respectively (Fig. 1). This results in overproduction of oxalate and L-glycerate and increased excretion of both metabolites in the urine.

Genetics

The disease is transmitted in an autosomal recessive mode. The *GRHPR* gene encoding for the glyoxylate reductase/hydroxypyruvate reductase is located on chromosome 9q11 [63]. Approximately 50 point mutations or small deletions have been identified, most of them involving the coding region or mRNA splicing [11, 61]. Two of these mutations, namely, c.103delG and c.403_404+2delAAGT, are more common. The former occurs exclusively in white populations, the latter almost entirely in Asian populations [64].

Clinical Presentation

Symptoms of PH2 usually develop in childhood (mean age at first symptom, 3.2 years; mean age at diagnosis, 8.0 years) and are characterized by recurrent stones, nephrocalcinosis, and ultimately CKD. Nephrolithiasis is found in 82.8% of patients [11]. Symptoms are comparable to PH1.

Diagnosis

The diagnosis of PH2 should be considered in every child with urolithiasis/nephrocalcinosis and in adults with recurrent CaOx stone events. In particular, the diagnosis of PH2 should be considered in the presence of hyperoxaluria without hyperglycolaturia, especially when *AGXT* mutation analysis is negative. In addition to hyperoxaluria and sometimes hyperglyceraturia (i.e., L-glycerate >5 μmol/L), the diagnosis of PH2 is based on the identification of pathogenic variants in the *GRHPR* gene.

Treatment and Outcomes

The initial management of PH2 patients relies on the reduction of urinary CaOx supersaturation through adequate daily fluid intake and treatment with inhibitors of CaOx crystallization (cf. PH1, Table 2). However, pyridoxine has no beneficial effect.

As in PH1 patients, oxalosis develops in patients with advanced CKD. The median kidney function survival in a very large cohort of 101 patients was 43.3 years [11]. In this cohort, 11 patients received a kidney transplant (12.3%), and 1 patient had undergone combined liver-kidney transplantation. There are only few other case reports of combined liver-kidney transplantation in PH2 patients that have experienced recurrence of the oxalate nephropathy after isolated kidney transplantation. Since the enzymatic defect is not restricted to the liver, combined liver-kidney transplantation is not systematically recommended in PH2. In the same large cohort of 101 patients, renal outcome did not correlate with genotype, biochemical parameters, or the presence of nephrocalcinosis at presentation [11].

Recently, RNA interference therapy with nedosiran, targeting the LDHA enzyme that mediates the final step of oxalate production (Fig. 1), has been shown to normalize Uox excretion also in PH2 [40]. Early treatment with nedosiran might preserve GFR or postpone kidney transplantation in patients that begin treatment at later stages of the disease.

Primary Hyperoxaluria Type 3

In 2010, mutations in the *HOGA1* gene have been shown to be responsible for a new type of PH, designated as PH3 [65].

Pathophysiology

PH3 (OMIM 613616) results from the loss-of-function of the mitochondrial enzyme 4-hydroxy-2-oxoglutarate aldolase (HOGA). HOGA catalyzes a lyase reaction in the final step of the hydroxyproline catabolism, leading to glyoxylate and pyruvate formation from hydroxy-2-oxoglutarate (HOG) (Fig. 1). Theoretically, loss of HOGA function should abrogate glyoxylate production and consequently should not cause hyperoxaluria, since glyoxylate is the immediate precursor of oxalate. Although the exact mechanism underlying PH3 physiopathology remains elusive, two explanations have been proposed. Both assume the existence of an alternative HOG-aldolase activity, which converts part of the accumulated HOG to glyoxylate. In one hypothesis, accumulation of HOG inhibits GRHPR activity, mimicking PH2 [66]. However, HOG inhibits significantly GRHPR only at millimolar concentrations, while nanomolar concentrations have been observed in liver samples obtained from patients with PH3. In addition, individuals with PH3 excrete normal amount of L-glycerate in their urine, which implies preserved cytosolic GRHPR activity. An alternative explanation is that HOG accumulates in mitochondria and is released into the cytosol, where it is converted to glyoxylate by an alternative cytosolic HOG-aldolase [67]. In the cytosol, glyoxylate can serve as substrate for both GRHPR and LDH. However, the characteristics of these enzymes and the oxidation state of their cofactors favor the preferential conversion of glyoxylate to oxalate by LDH [67].

Genetics

More than 50 *HOGA1* pathogenic variants have been detected in PH3 patients [68]. The most common variants are the c.700+5G>T splice site variant in intron 5, resulting in the in-frame insertion of 17 amino acids and the in-frame deletion c.944_946delAGG (p.Glu315del).

The estimated prevalence of PHs based on carrier frequency analysis suggests that PHs are more common than reported in clinical cohorts; this is particularly the case for PH3, which is predicted to have a similar prevalence than PH1 and to be twice as common as PH2 [4].

It has been suggested that PH3 heterozygous carriers are prone to kidney stone disease or have hyperoxaluria, but this view has recently been disputed.

Clinical Presentation

Compared to PH1 and PH2, the PH3 phenotype is less severe and is usually characterized by early-onset kidney stone disease without progression to CKD in most cases [65]. Same patients are diagnosed with a first stone episode only in their second or third decade of life. In addition, screening of sibling of patients with PH3 has identified subjects in their third decade of life with biallelic *HOGA1* pathogenic variants that have remained asymptomatic despite persistent hyperoxaluria [65, 67]. The discrepancy between the observed prevalence of the disease and the predicted prevalence from allele frequency studies confirms the milder PH3 phenotype, with many patients remaining undiagnosed.

Nephrocalcinosis is also much less common in PH3. Data from the Rare Kidney Stone Consortium PH registry indicate that nephrocalcinosis is present in 30% of PH1 patients at their first renal ultrasound examination and in 48% and 57% of patients after 10 and 20 years of follow-up, respectively. In comparison, only 7% of PH3 patients have nephrocalcinosis at their 5-year follow-up ultrasound, with no further increase at later investigations [69]. A recent analysis of the same registry has shown that stone events per decade of life was similar across the age span and the three types of PH [70].

Diagnosis

The diagnosis of PH3 should be considered in every child with kidney stone disease and in adults with recurrent CaOx stone events. Uox excretion rates are overlapping between the 3 forms of PH. It has been suggested that on average, patients with PH3 tend to have lower Uox levels, but higher urinary calcium and uric acid concentrations. Nonetheless, only 10% of PH3 patients have hypercalciuria at diagnosis [70]. The stones composition in PH3 patients is of pure CaOx monohydrate or a combination of CaOx mono- and dihydrate (weddellite) [70].

High urinary glycolic acid is characteristic of PH1 but has also been reported in PH3 patients [71]. Elevated urinary levels of HOG and of its derivative compound 4-hydroxyglutamate (4OHGlu) effectively identify patients with PH3 [67, 72]. It has been suggested that HOG is unstable at room temperature and may not represent a reliable biomarker for screening and diagnosis in this disease [72]. However, it has been used successfully in a number of laboratories. Moreover, 4OHGlu can be measured on newborn dried blood samples and may therefore be used for newborn screening [72].

The diagnosis of PH3 should always be confirmed by demonstrating pathologic variants in the *HOGA1* gene.

Treatment and Outcomes

There are no currently approved specific therapies for PH3. Conservative measures including adequate hydration and oral citrate supplements are important to increase CaOx solubility and prevent crystallization, similarly to what is recommended for PH1 (Table 2). In patients with hypercalciuria, low salt diet and thiazides are also recommended. Recommendations on dialysis and transplantation do not apply to PH3. As mentioned above, LDHA inhibition by siRNA (nedosiran) may represent an efficient and specific treatment in all types of PH. This approach is under investigation in PH3.

Some patients become asymptomatic overtime, despite persistent hyperoxaluria [73]. Only one PH3 patient progressing to ESKD has been reported to date; loss of kidney function in that patient was attributed, at least partly, to obstructive uropathy [4]. A recent analysis of the Rare Kidney Stone Consortium PH registry has shown that most PH3 patients maintain a normal GFR over time. Seven PH3 patients followed into their 40s and 50s have been reported in the registry, and only one has stage 3 CKD [70]. Three additional PH3 patients aged more than 60 years with CKD3 have also been reported. In comparison, 64% of PH1 patients and 34% of PH2 patients had reached ESKD at age 40 in the same database [70]. It has been suggested that obstructive kidney stone events in PH3 play an important role in the progression of kidney disease, but long-term follow-up studies engaging both pediatric and adult nephrologists are needed to investigate this hypothesis.

As mentioned above, some PH3 patients remain asymptomatic. The mechanisms that protect these patients from forming kidney stones despite significant hyperoxaluria are still unclear. Understanding these mechanisms may be very important to develop alternative therapeutic strategies for all patients with PH.

Secondary Hyperoxalurias

Oxalate in humans originates from the endogenous metabolism and from diet. Dietary oxalate is absorbed in the colon and distal portions of the ileum by passive diffusion and by active transport. Normal intestinal excretion of oxalates ranges 40 to 50 mg per day. The main dietary sources of oxalate include black tea, nuts, chocolate, and green leafy vegetables like spinach.

Enteric Hyperoxaluria

The principal cause of secondary hyperoxaluria is intestinal malabsorption, causing enteric hyperoxaluria. In this condition, the permeability of the colonic mucosa to oxalate is increased. In addition, fatty acids bind calcium, increasing the amount of free oxalate, which is more soluble and easily absorbed. Patients with intestinal malabsorption have a significant risk of stone disease that may cause CKD and even progress to ESKD [74]. Malabsorption disorders include inflammatory bowel diseases, chronic pancreatic insufficiency (including cystic fibrosis), ileal resection, short bowel syndrome, and celiac disease. Enteric hyperoxaluria can also be observed after bariatric surgery (mainly Roux-en-Y gastric bypass), in patients receiving unbalanced diets with restricted calcium intake, or after colon decolonization of bacteria that metabolize oxalate (lack of oxalate degrading intestinal bacteria *Oxalobacter formigenes* after wide-spectrum antibiotics, e.g., cystic fibrosis). Monitoring Uox is recommended in these conditions.

Abuse Intake of Oxalate Precursors

Excessive ascorbic acid supplements are metabolized to oxalate, which are excreted in urines. Supplements should not exceed 1000 mg per day. Vitamin C overload may represent an important issue in children on dialysis [35].

Ethylene glycol is a precursor of glycolic, glyoxylic, and oxalic acids. Poisoning with ethylene glycol may cause acute kidney and multisystemic injury, mainly in young children.

Patients with ESKD

In patients with ESKD, reliable measurement of Uox excretion is not possible [75]. Increased Pox levels up to 40 μmol/L may be observed in all forms of ESKD. Conversely, Pox concentrations above 50 μmol/L are highly suggestive of PH.

CaOx crystal deposition has been found in 4% of renal transplant biopsies [76], often because of acute tubular necrosis. Sometimes, however, these deposits are also present in the native kidneys, and in a small percentage of patients, undiagnosed PH1 or PH2 is revealed by this finding (Fig. 3). The final diagnosis requires molecular testing.

Food-Induced Secondary Hyperoxaluria

Some foods have a high oxalate concentration and can be responsible for secondary hyperoxaluria, mainly in patients with impaired GFR (star fruit, cashew nut, etc.) [77, 78].

Management

Hydration and crystallization inhibitors are as essential, similarly to patients with PH (Table 2). Specific measures are equally important, including treatment of the primary disease, dietary restriction of glyoxylate precursors, and promoting intestinal oxalate degradation with *Oxalobacter formigenes* [34, 50, 55]. Future developments include the

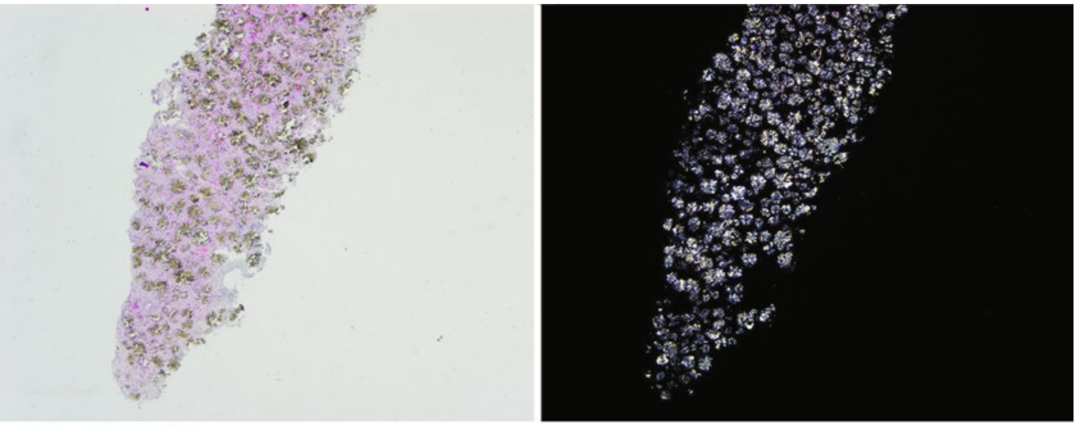


Fig. 3 Calcium oxalate deposition on bone biopsy in an adult PH1 patient with ESKD (x20) – left, hematoxylin eosin staining; right, polarized light microscopy. (Courtesy Dr N Dattner & Dr M Halfon, Lausanne, Switzerland)

use of oxalate-degrading enzymes (oxalate decarboxylase).

Conclusion

Hyperoxaluria are a group of rare heterogeneous diseases that are mostly inherited. The diagnosis should be considered in all children with a first stone episode and in adults with recurrent/bilateral nephrolithiasis, regardless of the presence of nephrocalcinosis or impaired kidney function. After excluding secondary hyperoxaluria, genotyping should be performed rapidly in order to confirm the phenotype and initiate appropriate management. Screening is recommended for all family members that are at risk, including prenatal diagnosis. Type 1 is the most common form of PH. In the past decades, most of these patients have undergone combined liver-kidney transplantation. New treatments have recently been introduced, in particular RNA interference therapies, which will most likely replace liver transplantation. Some of these treatments can also be beneficial in PH2 and PH3.

In the past years, PH has received significant attention. Guidelines for diagnosis have been produced, and new treatments have been developed. However, a large gap between high- and low-resource countries exists, which is made more relevant by the fact that the disease is most frequent, due to consanguinity, in countries with more limited medical resources. This raises unsolved ethical issues. Future efforts should focus on providing access to early screening, adequate diagnostic tools, genetic counseling, and effective therapies to patients from low-resource countries in order to avoid the need for organ transplantation, which is challenging for financial and technical reasons.

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Part V

Renal Tubular Disorders

Pediatric Fanconi Syndrome

36

Takashi Igarashi, Francesco Emma, and Wesley Hayes

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Abstract

Fanconi syndrome (FS) is generalized dysfunction of the renal proximal tubules leading to excessive urinary wasting of amino acids, glucose, phosphate, uric acid, bicarbonate, and other solutes. Patients also manifest renal salt wasting, hypokalemia, metabolic acidosis, hypercalciuria, and low-molecular-weight (LMW) proteinuria. Children with FS experience growth faltering, polyuria, polydipsia, dehydration, and rickets; adults suffer osteoporosis and osteomalacia.

De Toni, Debré, and Fanconi described children with renal rickets and glucosuria in the 1930s (De Toni, Acta Paediatr 16:479–84, 1933, Debré et al., Arch Med Enf 37:597–606, 1934, Fanconi, Jahrb Kinderheilkd 133: 257–300, 1931). FS is named after Guido Fanconi, a Swiss pediatrician, also known as "De Toni-Debré-Fanconi" syndrome.

Keywords

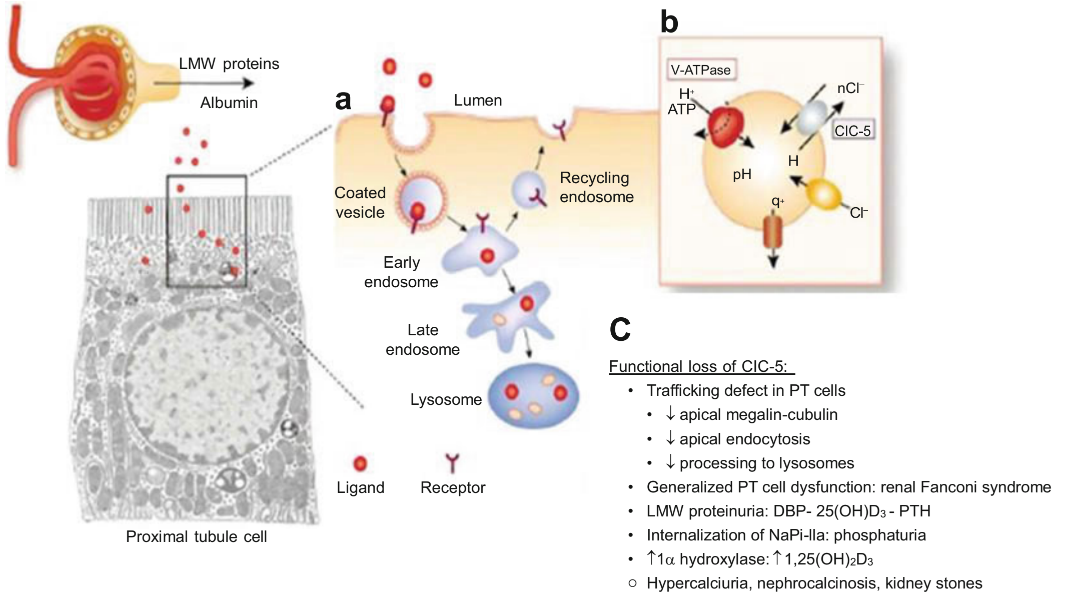
Fanconi syndrome · Generalized dysfunction of the renal proximal tubules

Pathophysiology

The renal proximal tubules reabsorb the majority of the glomerular filtrate including albumin, LMW proteins, amino acids, glucose, bicarbonate, sodium, chloride, phosphate, and uric acid. Most solute transport in the proximal tubule is mediated by receptor-mediated endocytosis or sodium gradient-dependent transport systems.

Receptor-Mediated Endocytosis

Proximal tubular cells have extensive luminal receptors and endocytic receptors such as megalin and cubam that are critical for the reabsorption, degradation, and recycling of proteins that have not been retained by the glomerular filtration barrier [1, 2] (Fig. 1). Cubam is composed of amnionless and cubilin [3]. Numerous filtered proteins including albumin, LMW proteins, polypeptide hormones, vitamin-binding proteins, and polybasic drugs such as aminoglycosides bind to megalin and cubilin in the luminal membrane of proximal tubules. The protein–receptor complex is subsequently incorporated into the endosome. The ligand and receptor are disassociated in the endosome; the receptor is recycled back to the luminal membrane and reabsorbed proteins traffic into the lysosome for further processing (Fig. 1). The disassociation of ligands from receptors is dependent on acidification of the endosome by increased concentration of H^+ and Cl^- that is mediated by the vacuolar-type H^+ -ATPase (V-ATPase) and the ClC-5 chloride channel. Disruption of the endocytic pathway compromises the recycling of megalin, cubilin, and



Functional loss of ClC-5:

- Trafficking defect in PT cells
  - ↓ apical megalin-cubulin
  - ↓ apical endocytosis
  - ↓ processing to lysosomes
- Generalized PT cell dysfunction: renal Fanconi syndrome
- LMW proteinuria: DBP- $25(OH)D_3$ - PTH
- Internalization of NaPi-IIa: phosphaturia
- ↑1α hydroxylase: ↑$1,25(OH)_2D_3$
- ○ Hypercalciuria, nephrocalcinosis, kidney stones

Fig. 1 Schematic model of endocytosis in the proximal tubule cells. Albumin and low-molecular-weight (LMW) proteins are filtered into the primary urine and endocytosed by proximal tubule cells via the megalin–cubilin receptor pathway. (**a**) The receptor–ligand complexes progress along the endocytic pathway. The endosomes undergo a progressive, ATP-dependent acidification that results in the dissociation of the receptor–ligand complexes, with megalin and cubilin being recycled in the luminal membrane, whereas the ligand is directed to lysosomes for degradation. (**b**) Vesicular acidification is mediated by the vacuolar H^+-ATPase, which requires a net Cl^- conductance to functions as an electrogenic nCl^-/H^+ exchanger, which is predicted to facilitate acidification and to play a role in keeping high vesicular Cl^- concentration. (**c**) Patients with Dent disease and ClC-5-null mice have multiple trafficking defects in PT cells, with (i) loss of megalin and cubilin in the apical brush border and their accumulation in subapical vesicles; (ii) defective apical endocytosis and low-molecular-weight proteinuria; and (iii) impaired progression toward lysosomes. These modifications result in generalized dysfunction of PT cells and renal Fanconi syndrome. As vitamin D-binding protein carries most of the circulating vitamin D in the plasma, its urinary loss may lead to bone defects and loss of $25(OH)D_3$ substrate. Increased amount of bioactive PTH in the PT lumen may interact with apical PTH receptors and stimulate internalization and degradation of the apical NaPi-IIa cotransporter, leading to phosphaturia. In turn, the phosphate loss could stimulate the 1α-hydroxylase activity in PT cells, leading to increased $1,25(OH)_2D_3$ synthesis depending on substrate availability. The balance between these effects may result in phenotype variations observed in man and mouse [4].

other transporters to the luminal membrane, causing decreased solute reabsorption. Altered endosomal acidification in proximal tubule cells decreases reabsorption and causes urinary wasting of albumin, LMW proteins, electrolytes, and solutes. For example, cadmium inhibits vacuolar acidification and mitochondrial function, which results in renal FS. Folimycin (concanamycin A), a H^+-ATPase inhibitor, abolishes albumin uptake by proximal tubules. Mutations in the ClC-5 channel in Dent disease can also cause FS [4]. In healthy children, megalin shed from proximal tubular membranes can be detected in urine. Conversely, almost no megalin is detected in the urines of patients with Dent disease, due to impaired recycling to the plasma membrane [5]. Similar results are observed in Lowe syndrome [5].

Sodium-Dependent Reabsorption

Reabsorption of filtered solutes including glucose, phosphate, amino acids, and bicarbonate by proximal tubular (PT) cells is accomplished by transport systems at the luminal brush border membrane that are directly or indirectly coupled to sodium transport. Mitochondria provide most of the energy required by the Na^+, K^+-ATPase, which maintains the electrochemical gradient allowing sodium-coupled entry of solutes into PT cells. Although kidneys make up less than 1% of total bodyweight, they consume approximately 10% of total energy in resting conditions. Transport processes consume most of the energy produced by PT cells. Usually, significant defects in PT energy production cause nonselective impairment of most transport mechanisms, resulting in FS. The mitochondrial respiratory chain has a major role in ATP production in the proximal tubule. Genetic defects that directly or indirectly alter the respiratory chain, as well as drugs and toxic substances that inhibit oxidative phosphorylation in PT cells can cause renal transport anomalies and FS [6, 7]. This is in contrast to isolated defects of apical transporter proteins resulting in selective losses of amino acids, glucose, phosphate, bicarbonate, or uric acid. Glucosuria or generalized aminoaciduria can be observed in some patients with selective apical transport defects, but these are usually modest. For example, some patients with renal glucosuria resulting from mutations in the kidney-specific low-affinity/high-capacity Na^+/glucose cotransporter gene (*SLC5A2*, 16p11.2) or with maturity-onset diabetes of the young type 3 (MODY3) caused by mutations in hepatocyte nuclear factor-1α gene (*HNF1A*, 12q24.31) that regulates transcription of *SLC5A2* [8, 9] may present with generalized aminoaciduria.

Signs and Symptoms

Growth Faltering

Growth faltering is a common feature of FS in children. Patients can present with severe growth faltering that may persist into adult life. The physiopathology of growth faltering in FS is complex. Malnutrition, hypokalemia, hypophosphatemia, and metabolic acidosis can all contribute [10].

Potassium deficiency reduces circulating levels of growth hormone (GH) and of insulin-like growth factor I (IGF-I). Hypokalemia can also decrease appetite, leading to malnutrition and extracellular volume contraction. Metabolic acidosis inhibits GH secretion and expression of IGF-I and of GH receptors [11]. Hypophosphatemia and impaired 1-alpha hydroxylation of 25-dihydroxyvitamin D lead to rickets [12]. In patients with adult-onset FS, renal phosphate wasting, and relative 1,25-dihydroxyvitamin D3 deficiency, hypophosphatemia is thought to cause osteomalacia [13]. The FS-associated metabolic acidosis also impairs 1-alpha hydroxylation. Some patients complain of bone and joint pain, and can suffer fractures. Decreased mineralization of the dentin structure and abnormal formation of craniofacial bones are also reported [14]. In forms of FS that are associated with defects in receptor-mediated endocytosis, low uptake of vitamin D and of its binding protein can aggravate metabolic bone disease [15].

Early diagnosis and treatment of acidosis and electrolyte imbalances promote growth and improve final height in children with FS. However, normal height is not often achieved, in particular in patients with cystinosis [16]. This may be related to a disease-specific bone defect.

Polyuria, Polydipsia, and Dehydration

Polyuria, polydipsia, and dehydration are frequently observed. Polyuria is primarily secondary to osmotic diuresis resulting from excessive urinary solute losses, compounded by impaired urinary concentration related to chronic hypokalemia and nephrocalcinosis. Recurrent episodes of fever due to dehydration are reported in infants [16].

Generalized Aminoaciduria

Amino acids are freely filtered by the glomerulus. Approximately 95–99% of the filtered load are reabsorbed by PT cells, which express specialized transporters in their apical and basolateral membranes. Except for histidine, which has a fractional excretion up to 5%, the fractional excretion of other amino acids is $<3\%$ in children after the neonatal period. Aminoaciduria (or hyperaminoaciduria) is therefore defined as a fractional excretion of amino acids >5%. In FS, aminoaciduria is generalized (i.e., nonselective).

$$\text{Fractional excretion of amino acid }(\%) \\ = [(\text{Uaa}/\text{Saa})/(\text{Ucr}/\text{Scr})] \times 100\%$$

(aa: amino acid; cr: creatinine; U: urine; S: serum)

Glucosuria

Glucosuria is a common manifestation in FS and is secondary to impaired reabsorption of glucose. Normally, the filtered load of glucose (D-glucose, MW = 180 D) is almost completely reabsorbed by sodium-coupled transport in the proximal tubule. Glucose reabsorption involves transporters at the luminal and basolateral membranes of the proximal tubules. Thus, a very small amount of glucose is present in the urine in health. Typically, the blood glucose level is normal. Glucosuria is one of the originally described clinical features of FS [17–19]; patients can lose 0.5–20 g/day of glucose in their urine.

Hypophosphatemia

Some patients with FS have reduced tubular reabsorption of phosphate (percent tubular reabsorption of phosphate: %TRP, >80–85% in healthy subjects) in combination with low serum phosphate concentration. Rickets and osteomalacia result from urinary phosphate wasting and impaired 1α-hydroxylation of 25-hydroxyvitamin D3 by proximal tubule cells [20]. The maximal threshold of phosphate (TmP/GFR) is a sensitive indicator of phosphate reabsorption. The Tm/GFR is usually inappropriately low in patients with FS (normal values 2.3–4.3 mg/dl). Other markers of bone chemistry such as PTH and alkaline phosphatase may be elevated in patients with FS. Serum 1,25 $(OH)_2D_3$ is variable [21].

Rickets usually manifests in small children with bowing deformity of the legs, wrist widening, and prominence of the costochondral junction, also termed “rachitic rosary.” Other signs include increased tendency for fractures, skull bossing, delayed fontanelle closure, craniotabes (soft skull), and Harrison’s groove.

$$\text{TRP }(\%) = [\,1 - (\text{Up}/\text{Sp})/(\text{Ucr}/\text{Scr})] \times 100$$

(p: phosphate; cr: creatinine; U: urine; S: serum)

$$\text{TmP}/\text{GFR} = \text{TRP} \times \text{Sp}$$

(GFR: glomerular filtration rate; p: phosphate; S: serum)

Metabolic Acidosis

More than 85% filtered bicarbonate (HCO_3^-) is reabsorbed by the PT cells, which is accomplished by the coordinated function of luminal membrane Na^+/H^+ exchanger, luminal membrane carbonic anhydrase IV and XIV, and basolateral membrane Na^+/HCO_3^- cotransporter. Defective bicarbonate reabsorption in the proximal tubules results in hyperchloremic metabolic acidosis and is a common feature of FS. The anion gap is normal. In overt forms of FS, more than 30% of the filtered load of HCO_3^- is not reabsorbed; patients have low plasma HCO_3^- levels. In patients with FS receiving alkali supplementation, fractional excretion of HCO_3^- ($FEHCO_3^-$) usually exceeds 15%. Urinary acidification in the distal tubule is usually normal, but can be sometimes impaired due to chronic hypokalemia or combined proximal-distal disease/toxicity.

Sodium and Potassium Losses

Approximately 60–80% of filtered sodium is normally reabsorbed in the proximal tubules. Sodium reabsorption is decreased in patients with FS. This may cause hypotension and dehydration. Hypokalemia is secondary to increased delivery of sodium to the distal segments and activation of the renin-angiotensin-aldosterone system as a result of hypovolemia. Potassium wasting may cause severe hypokalemia.

Hypercalciuria

Hypercalciuria is commonly observed in FS. In patients with heavy LMW proteinuria, such as in Dent disease, different mechanisms may counteract each other. Defective endocytosis of parathyroid hormone (PTH) increases its expression on the cell surface of proximal tubular cells, where it stimulates $25(OH)D_3$ 1-hydroxylase to produce more $1,25(OH)_2D_3$, raising serum levels of this vitamin. Conversely, patients lose vitamin D3-binding protein in urine, which binds $25(OH)D_3$ and presents it to the enzyme. The final levels of $1,25(OH)_2D_3$ depend on the balance of these opposing processes. Often, patients with FS have slightly elevated serum levels of $1,25(OH)_2D_3$, which increases intestinal calcium reabsorption resulting in absorptive hypercalciuria [21]. Hypercalciuria is rarely associated with nephrolithiasis in patients with FS, possibly because polyuria and alkalized urine mitigate calcium crystallization. Patients with Dent disease, however, can present with hypercalciuria and nephrolithiasis.

Hyperuricosuria

Uric acid (urate) is the end product of purine metabolism in humans. Because of its small molecular size (MW = 126 D), uric acid is freely filtered by the glomerulus. Approximately 90–95% of the filtered load of uric acid is reabsorbed in the proximal tubules. A four-component hypothesis has been proposed to explain renal uric acid transport; it includes glomerular filtration, presecretory reabsorption, secretion, and post-secretory reabsorption [22]. Hyperuricosuria is often present in FS, leading to secondary hypouricemia (<2 mg/dL). A voltage-sensitive uric acid pathway and uric acid exchangers are located at both luminal and basolateral membranes of proximal tubule cells. Uric acid reabsorption from the proximal tubule lumen into the cell is mediated by uric acid (urate)–anion exchanger (URAT1) and the glucose transporter 9 short isoform (GLUT9S) [23, 24]. *SLC2A9* produces two GLUT9 isoforms by alternative splicing; the long isoform (GLUT9L) is expressed in basolateral membrane of proximal tubular cells and the short isoform (GLUT9S) is expressed in apical membrane of proximal tubule cells [25]. The residual apical uptake of uric acid is probably mediated by OAT4 and OAT10 [26, 27]. Uric acid efflux from PT cells across the basolateral membrane appears to be primarily mediated by GLUT9L. GLUT9 was identified by screening genes associated with gout and hyperuricemia [28]. Defects in URAT1 or GLUT9 cause renal hypouricemia; patients can manifest AKI after exercise [29].

Low-Molecular-Weight Proteinuria

The PT reabsorb most proteins filtered by the glomerulus. The glomerular molecular weight cut-off for plasma proteins is approximately 65 KD (kilodaltons), similar to the molecular weight of serum albumin. A small amount of larger molecular weight proteins, including gamma globulin, can also pass the glomerular filter in normal conditions. It has been estimated that up to 9 g of albumin may be filtered every day in healthy humans [30]; of these, less than 0.1–0.15 g/day are excreted in urine.

Abnormal proteinuria can result from increased glomerular leak, defects in tubular reabsorption of proteins, or both. In FS, excess proteinuria is primarily caused by impaired reabsorption. For example, adolescent patients with FS secondary to Dent disease and Lowe syndrome excrete large amounts of proteins (1740 ± 660 mg/day) and small peptides (446 ± 145 mg/day) [31]. In patients with FS, small peptides ranging 2–5 KD are present more than 10-fold in excess, compared with normal urine [31].

Various structurally different filtered proteins, including albumin and most LMW proteins, bind to megalin and cubilin, in the luminal aspects of proximal tubules. Protein–receptor complexes are then internalized into endosomes, where they dissociate. The receptor is recycled back to the plasma membrane, while the ligand is directed towards the lysosomal compartment, where it is further processed. Megalin is a 600 KD glycoprotein that contains a large amino-terminal, an extracellular domain, a single transmembrane domain, and a short carboxy-terminal cytoplasmic tail; it is a member of the low-density lipoprotein receptor family. Megalin is expressed in the proximal tubule brush border and luminal endocytic apparatus. Cubilin is a 460 KD multiligand endocytic receptor with little structural homology to other known proteins. It is expressed in the proximal tubule brush border and luminal endocytic apparatus. Megalin has a primary role in reabsorbing filtered albumin both directly, as a receptor for albumin, and indirectly by promoting the expression of cubilin [32]. Patients with Dent disease have decreased expression of megalin. The acidification defect secondary to impaired chloride conductance alters recycling of megalin and cubilin from the endosomal compartment to the plasma membrane.

Decreased Urinary Excretion of Osteopontin and Uromodulin

Osteopontin is excreted into the urine from renal tubule cells. It plays a role in remodeling of extracellular matrix and inhibition of apoptosis, and as a protective factor against nephrocalcinosis. Uromodulin (Tamm-Horsfall protein) is also excreted by renal tubule cells and plays a role in combating urinary tract infections. Proteome analysis revealed that urinary excretion of both proteins is reduced in patients with FS [33].

Causes of FS

Causes of FS can be divided into hereditary, acquired, and exogenous toxins (Table 1). Most hereditary FS occurs in the context of congenital inborn errors of the metabolism or, more rarely, as sporadic or familial disorders. Acquired forms are mostly secondary to immunological disorders, nephrotic syndrome, or accumulation of abnormal proteins in renal tubules. Exogenous substances include drugs, chemical compounds, and heavy metals.

Table 1 Causes of Fanconi syndrome

Hereditary
Dent disease
Lowe syndrome
Mitochondriopathies
Enoyl-coenzyme A hydratase/L-3-hydroxyacyl-coenzyme A dehydrogenase
EHHADH deficiency
Cystinosis
Galactosemia
Hereditary fructose intolerance
Glycogen storage disease type 1 (von Gierke disease)
Fanconi-Bickel syndrome
Tyrosinemia type I
Wilson disease
Lysinuric protein intolerance
Microvillus inclusion disease
Maturity-onset diabetes mellitus of the young (MODY)
Type IIa sodium/phosphate cotransporter (NaPi-IIa) deficiency
Arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome
Idiopathic
Acquired
Nephrotic syndrome
Myeloma
Sjögren syndrome
Renal transplantation
Acute tubulointerstitial nephritis and uveitis (TINU) syndrome
Autoimmune interstitial nephritis and membranous nephropathy
Anorexia nervosa
Untreated condition of distal renal tubular acidosis
Exogenous substances
Drugs
Chemical compounds
Heavy metals

Hereditary Causes of Fanconi Syndrome

Cystinosis

See ▶ Chap. 37, "Cystinosis"

Dent Disease

Dent disease is an X-linked proximal tubulopathy characterized by LMW proteinuria, hypercalciuria, nephrocalcinosis, and progression towards end-stage kidney disease. Hypophosphatemic rickets, hypokalemia, metabolic acidosis, and nephrolithiasis (calcium oxalate and/or phosphate) are often observed [34, 35]. Almost all patients are males. Most adult patients have FS. Children manifest with LMW proteinuria and usually one or two additional signs of PT dysfunction (partial FS) [36]. Extrarenal symptoms are rare, with the exception of rickets. In Japan, a school screening program identified asymptomatic children with Dent disease [37].

Dent disease is genetically heterogeneous. In approximately 60% patients, Dent disease is caused by inactivating mutations of the *CLCN5* gene (Xp11.23), which encodes for a 746 amino acid chloride channel [38]. ClC-5 is exclusively expressed in the kidney. The disease is termed more specifically Dent disease 1 (DD1), to differentiate it from similar phenotypes caused by *OCRL1* mutations [see below, Dent disease 2 (DD2)]. ClC-5 belongs to the family of voltage-dependent chloride channels, which function as homodimeric proteins. It is co-expressed with the V-ATPase and plays a key role in the endosomal acidification process, which is a critical step in the receptor-mediated endocytic pathway [39]. There is no clear genotype–phenotype correlation; a large degree of variation in phenotype is observed between affected members of the same family [37]. Carrier females have significantly less severe LMW proteinuria and hypercalciuria; some with unfavorable X-chromosome inactivation may develop clinically significant symptoms.

Abnormal endocytosis secondary to *CLCN5* mutations disturbs normal recycling of megalin and cubilin to the luminal membrane, causing LMW proteinuria, and alters the expression of other transporter proteins, causing partial or complete FS. LMW proteins undergo receptor-mediated endocytosis in proximal tubules (see above). Chloride-influx via ClC-5 facilitates endosomal acidification by maintaining electroneutrality of ion transport. The absence or dysfunction of ClC-5 disrupts endosome recycling in the PT cells by reducing receptor internalization, altering progression of endosomes to lysosomes and disrupting recycling of endosomes to the cell surface [40].

In most cases, excess proteinuria ranges from 0.5 to 2.5 g per day but may exceed 4 g in some patients. Proteins with a molecular weight <45 KD represent approximately 60% of the filtered protein load. LMW proteinuria is a consistent feature of Dent disease in all patients. Markers of LMW proteinuria, such as β2-microglobulin (MW = 11.6 KD), are excreted in the urine in amounts reaching 100–300 times the upper limit of the normal value. Excess albumin (MW = 65 KD) is also lost in urine, but not in quantities causing hypoalbuminemia and nephrotic syndrome. This pattern of proteinuria with predominant excretion of excess LMW proteins is termed tubular proteinuria and has been used interchangeably with “LMW proteinuria.”

Impaired urinary concentrating ability is frequently observed. Reduced expression of sodium hydrogen exchanger 3 (NHE3) in PT cells may also contribute to polyuria by increasing sodium losses.

The pathophysiology of hypercalciuria is complex. Overall, 70% of patients with *CLCN5* mutations have hypercalciuria. Nephrocalcinosis is a frequent finding (Fig. 2), but it is not present in all patients. Urinary calcium excretion is usually in the range of 4–10 mg/kg/day in affected children and 4–6 mg/kg/day in adults [41]. Vitamin D metabolism is subject to opposing effects resulting from decreased recycling of the PTH receptor to the luminal membrane of PT cells, and increased urinary losses of vitamin D-binding protein. Slightly elevated serum levels of $1,25\text{-}(OH)_2D_3$ are often observed in patients with Dent disease and can lead to increased intestinal Ca^{2+} reabsorption and hypercalciuria. Expression of ClC-5 in other nephron segments may also play an important role in the pathophysiology of hypercalciuria. In vitro, inactivation of the *CLCN5* gene in collecting duct cells promotes membrane retention of annexin 2, which facilitates formation of calcium-based crystals on the cell surface and may explain the nephrocalcinosis observed in many patients [42]. Decreased urinary excretion of osteopontin, a protective factor against nephrocalcinosis, can also play a role. Nephrolithiasis occurs in about 25% patients.

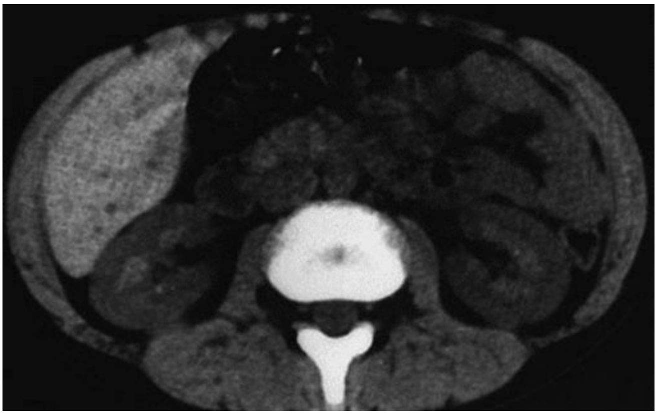


Fig. 2 Abdominal CT demonstrating bilateral medullary nephrocalcinosis in a 12-year-old boy with Dent disease

Hypophosphatemia secondary to renal phosphate losses is observed in almost half of adult patients with DD1. Phosphate is reabsorbed in the proximal tubules by the NaPi-IIa and NaPi-IIc proteins (type IIa and IIc sodium/phosphate cotransporters). In ClC-5 knockout mice, altered apical endocytosis of filtered PTH decreases apical expression of NaPi2a, leading to urinary phosphate wasting [43].

In the collecting duct, ClC-5 colocalizes with V-ATPase in apical and subapical vesicles. Decreased ClC-5 function probably disturb recycling of these vesicles leading to defective apical expression of the V-ATPase in collecting ducts and impaired urinary acidification.

Kidney function in patients with Dent disease is highly variable. Some progress to end-stage kidney disease, typically in the fifth to sixth decades of life; one-third of patients do not progress to end-stage kidney disease; some develop significantly impaired kidney function in childhood. Kidney biopsy has no diagnostic role. Histology shows normal or focal global glomerulosclerosis with tubular atrophy, tubular dilatation, and interstitial infiltration of inflammatory cells. The underlying mechanisms leading to end-stage kidney disease in DD1 are incompletely understood. Medullary nephrocalcinosis may contribute. High urinary concentrations of bioactive proteins including insulin, insulin-like growth factor-1 (IGF-1), and the chemokine monocyte chemoattractant protein-1 (MCP-1) may contribute to interstitial fibrosis. Proximal tubule dysfunction is associated with cell dedifferentiation and oxidative stress.

Pathogenic variants in the *OCRL1* gene (Xq26.1) have been identified in approximately 15% of patients with a Dent phenotype [44]. This phenotype is referred to as Dent disease 2 (DD2). Approximately 25% of the patients with Dent disease remain genetically unresolved. Pathogenic variants in *OCRL1* usually cause Lowe syndrome; unlike patients with typical Lowe syndrome, patients with DD2 have normal or near normal facial appearance, intelligence, muscle tone, and ocular findings. Their phosphoinositol 4,5-bisphosphate phosphatase (PIP_2 5-phosphatase) activity is markedly reduced in skin fibroblasts and protein expression is reduced or absent. This enzyme regulates endosomal trafficking and recycling, causing LMW proteinuria if mutated (see below). Unlike patients with Lowe syndrome, patients with DD2 usually do not develop metabolic acidosis. Similarly to Lowe syndrome, however, their lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) levels are frequently increased, a sign that may orient the initial genetic testing. Also, glycosuria is very rarely reported with OCRL1 variants. Phenotypic differences between DD2 and Lowe syndrome are not explained by the severity of OCRL1 variants. However, nearly all variants associated with Lowe syndrome are located in exons 8–23, whereas the majority of pathogenic variants for DD2 are located in exons 1–7 and those that are located after exon 7 are always missense mutations [146].

There is currently no specific treatment to protect kidney function in Dent disease. Thiazides have been administered to mitigate hypercalciuria, aiming to preserve kidney function

[45, 46]. However, no long-term study has provided evidence that this treatment delays the natural history of progression. Moreover, patients developed adverse reactions, including muscle cramps, hypovolemia, hypokalemia, and hyponatremia [46]. A high citrate diet delayed progression of nephrocalcinosis and of chronic kidney disease in *clcn5* knockout mice [47]. Treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) may also delay progression [48]; however, these treatments should be used with caution in patients with urinary concentration defects. Animal data in a mouse model of Dent disease suggested that bone marrow transplantation could rescue the epithelial phenotype of the proximal tubules [49].

Lowe Syndrome

Lowe oculocerebrorenal syndrome is a rare X-linked disorder caused by variants in the OCRL1 gene (Xq26.1). The syndrome is characterized by a complex phenotype that includes severe eye abnormalities, central nervous system abnormalities, and FS [50, 163].

Cataracts are recognized in all male patients. Lens opacities are present in all carriers of females by slit-lamp examination and has been used to screen for carriers [51]. Other ocular abnormalities include glaucoma, microphthalmos, and corneal keloid formation. Visual acuity is disturbed. Central nervous system abnormalities include infantile hypotonia (floppy infant), areflexia, and developmental delay. Ventriculomegaly and periventricular cysts may be seen. Some patients experience seizures including status epilepticus. FS is a major clinical feature and commonly occurs in the first year of life, but the severity and age of onset vary. All patients manifest LMW proteinuria. Aminoaciduria, hyperphosphaturia, hypercalciuria, hypophosphatemic rickets, hyperchloremic metabolic acidosis are frequently reported, while glucosuria is rare [61, 147]. All patients develop progressive renal insufficiency and typically progress to end-stage kidney disease by the 30s or 40s [52]. Compared to patients with DD2, patients with Lowe syndrome demonstrate faster decline in kidney function [147].

Megalin is nearly absent from urine in patients with Lowe syndrome, indicating decreased megalin expression in the luminal membrane of the proximal tubule cells, while *N*-acetylglucosaminidase is significantly increased, indicating urinary shedding of lysosomes [53].

The *OCRL1* gene (Xq26.1) encodes an inositol polyphosphate 5-phosphatase [54, 146]. This enzyme is a predominantly lipid phosphatase that regulates cell levels of phosphatidylinositol 4,5-bisphosphate, which is a critical metabolite of the inositol phosphatase signaling pathway [55, 146]. It is expressed in skin fibroblasts, but not present in peripheral blood cells. Its activity is markedly reduced in fibroblasts from patients with Lowe syndrome [56], but the test cannot reliably detect female carriers.

Deficiency of inositol polyphosphate 5-phosphatase OCRL leads to cellular accumulation of its substrate PIP2 (Phosphatidylinositol (4,5)-bisphosphate) [57]. PIP2 is involved in signal transduction, vesicle trafficking, and actin polymerization. Absence of PIP2 5-phosphatase activity impairs actin metabolism, altering several epithelial functions, including cell–cell interactions, clathrin-mediated endocytosis, trafficking of receptors through early endosomes, recycling of proteins to the plasma membrane, retrograde transfer to the Golgi complex, and sorting of cargo to lysosomes [58, 59, 146]. In addition, PIP2 accumulates in autolysosomes causing defective autophagic flux [146]. Lack of OCRL causes abnormal morphology of the primary cilium in several cells types [146]. Finally, *OCRL1* has a C-terminal RhoGAP domain that binds the Rac GTPase. Altered Rho-dependent pathways may play an important role in developmental delay in Lowe syndrome, as observed in other forms of X-linked developmental syndromes [60].

Many *OCRL1* variants have been described in patients with Lowe syndrome; nearly all are clustered in exons 10–23, especially exon 15, and almost none are found in exons 1–9 [56, 147].

Treatment is supportive and includes care of ocular manifestations and dental complications, seizures, and speech therapy. Cataracts should be treated early. Usually patients require supplementation with bicarbonate, sodium, or potassium phosphate, and vitamin D; requirements vary significantly from patient to patient.

Life expectancy is reduced in patients with Lowe syndrome to approximately 30–40 years. End-stage kidney disease is the most frequent cause of death. Some patients have been treated with dialysis, but behavioral instability, in particular aggressive behavior, often represents an important hurdle to successful kidney replacement therapy. A minority of patients have undergone kidney transplantation.

Mitochondrial Diseases

Since PT cells are metabolically very active and dependent heavily mitochondria for their energy supply, many mitochondrial cytopathies can cause FS [153] (see ▶ Chap. 33, "The Kidney in Mitochondrial Diseases").

Most patients with FS secondary to mitochondrial cytopathies present with complete FS, usually before the age of 2 years, in combination with extrarenal symptoms, mostly neuromuscular. In some cases, the FS is incomplete. Kidney biopsy, if performed, often shows cytoplasmic vacuolization of tubular cells with tubular dilatation and atrophy. Abnormal mitochondria are frequently observed by electron microscopy.

Treatment of the FS is mainly symptom-based and includes supplementing sodium bicarbonate, potassium, vitamin D3, phosphate, and fluid as needed. Drugs that interfere with the respiratory chain, such as valproate and barbiturates, or that inhibit mitochondrial protein synthesis, such as tetracyclines and chloramphenicol, should be avoided.

Enoyl-Coenzyme A Hydratase/L-3-Hydroxyacyl-Coenzyme A Dehydrogenase (EHHADH) Deficiency

Some variants in the *EHHADH* gene (3q27.2), which encode EHHADH (enoyl-coenzyme A hydratase/L-3-hydroxyacyl-coenzyme A dehydrogenase), can lead to FS. Patients present with rickets, impaired growth, glucosuria, generalized aminoaciduria, phosphaturia, metabolic acidosis, and LMW proteinuria [62].

EHHAD is a peroxisomal protein involved in β-oxidation of fatty acids. Variants at p.E3K result in a de novo mitochondrial import sequence, resulting in mistargeting to mitochondria. The mutated EHHADH protein is incorporated into mitochondrial trifunctional protein, thereby disturbing β-oxidation of long-chain fatty acid and mitochondrial energy production, which is predominantly based on fatty acid oxidation (FAO) in proximal tubular cells, leading to FS [63].

Galactosemia

Galactosemia is an autosomal recessive disorder affecting galactose metabolism. In nursing infants, large amounts of galactose are processed through the Leloir pathway in order to utilize carbon for energy [64]. Classic galactosemia is due to deficient activity of galactose-1-phosphate uridyltransferase (GALT) enzyme encoded by the *GALT* gene (9q13.3) [65]. GALT catalyzes the metabolism of galactose-1-phosphate (gal-1-p) plus uridine diphosphate glucose to uridine diphosphate galactose plus glucose-1-phosphate (Fig. 3). Uridine diphosphate galactose can be further metabolized to either glucose or CO_2 and H_2O via glycolysis. Milk is a major source of galactose. Accumulated gal-1-p due to defective GALT and exposure to galactose leads to acute metabolic deterioration in multiple organ systems, including liver, kidney, ovary, brain, and eye. GALT deficiency results in accumulation of toxic amount of galactose leading to unfolding of proteins, altered calcium homeostasis, and endoplasmic reticulum (ER) stress [66]. ER stress caused by GALT deficiency may contribute to accelerated apoptosis of follicular cells, leading to premature ovarian failure in females [67]. Abnormal synthesis of galactitol from galactose through aldose reductase probably represents a key element in the pathophysiology of cell

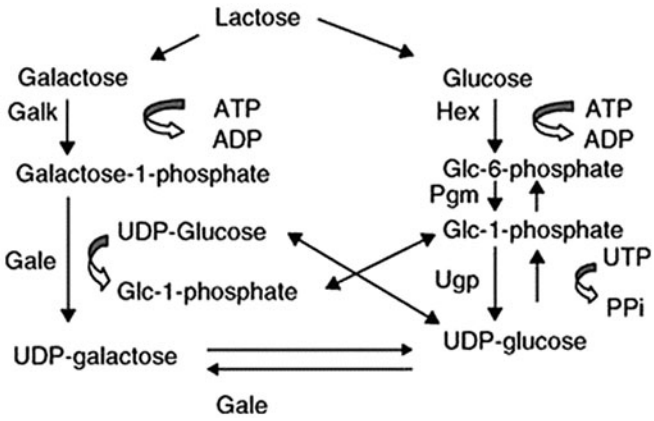


Fig. 3 Composite diagram of the Leloir pathway and uridine diphosphate (*UDP*)–glucose pyrophosphorylase pathway. *Galk* galactokinase, *galt* galactose-1-phosphate uridyltransferase, *Gale* UDP-galactose 4-epimerase, *Hex* hexokinase, *Pgm* phosphoglucomutase, *Ugp* UDP-glucose pyrophosphorylase [64]

damage in galactosemia and is responsible for the formation of cataracts.

Affected infants manifest vomiting, diarrhea, growth faltering, developmental delay, liver dysfunction, coagulopathy, renal tubular dysfunction, cerebral edema, vitreous hemorrhage, and sepsis (mostly *Escherichia coli*). They sometimes develop jaundice with unconjugated hyperbilirubinemia and may suffer from severe hemolysis. Liver damage leads to hepatomegaly and cirrhosis that can be lethal. Thanks to neonatal screening programs [71], most patients are diagnosed very early, which allows minimizing long-term complications, such as developmental delay. Patients placed on a galactose-restricted diet, however, are never truly free of galactose, as a significant amount is found in nondairy foodstuffs such as vegetables and fruits [68]. More importantly, galactose moieties can also be produced endogenously from UDP-glucose via the UDP-4-galactose epimerase reaction during the natural turnover of glycoproteins and glycolipids [69]. Therefore, even well-treated patients can develop debilitating complications [70]. Growth retardation, speech delay, and cerebellar symptoms are common. Premature ovarian failure nearly always develops in females. The most frequent renal finding is FS.

Treatment involves elimination of galactose from the diet. Acute symptoms and signs resolve within a few days of starting dietary restriction.

Hereditary Fructose Intolerance

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder caused by a deficiency in the liver, the intestine, and renal cortex of aldolase B (fructose-1-phosphate aldolase), which catalyzes breakdown of exogenous fructose [72]. The frequency of HFI is estimated at 1 in 20,000 live births. Aldolase B is encoded in *ALDOB* gene (9q31.1) [73]. It cleaves fructose-1,6-bisphosphate (FBP) and fructose-1-phosphate (F1P) into dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate, or into dihydroxyacetone phosphate and D-glyceraldehyde, respectively.

Shortly after the intake of fructose or related sugars, including sucrose and sorbitol, affected individuals manifest symptomatic hypoglycemia, vomiting, restlessness, which can progress to life-threatening shock [74]. Protracted ingestion of fructose causes growth faltering, hepatomegaly, jaundice, hepatic cirrhosis, and nephrocalcinosis, and may lead to convulsions, coma, and death from severe liver and kidney failure. Symptoms of HFI appear during infancy when infants start solid food including fruit, vegetables, and sweetened foods that contain sucrose. Current formulae are no longer sweetened with fructose or sucrose. Patients with HFI often develop aversion to sweets and fruits.

At the renal level, HFI causes PT dysfunction leading to aminoaciduria, bicarbonaturia, and phosphaturia. These changes occur rapidly after ingestion of fructose [72, 74]. Development of lactic acidosis adds significantly to the metabolic acidosis [75]. Chronic fructose ingestion leads to nephrocalcinosis and impaired distal tubular function. In contrast, resolution of proximal tubule dysfunction occurs within days or weeks after strict dietary restriction of fructose and sucrose.

Aldolase B is abundantly co-expressed in endocytic structures of the PT cells, together with H^+-ATPase [76]. The pathophysiology of FS in HFI is not clearly understood but may involve, at least in part, defective endosomal acidification.

The diagnosis is confirmed by molecular genetic testing, which has largely replaced previous biochemical investigations. Strict avoidance of foods and drugs containing fructose, sucrose, and sorbitol is the mainstay of treatment.

Glycogen Storage Disease Type I

Glycogen storage disease type I (GSD-I, von Gierke disease) refers to a group of autosomal recessive disorders with an incidence of 1 in 100,000. There are two major subtypes [151, 152]. Glycogen storage disease type Ia (GSD-Ia) is common (80%) and is caused by the mutation in *G6PC* gene (17q21.3) resulting in the deficiency of glucose-6-phosphatase-alpha (G6Pase-alpha), a key enzyme in glucose homeostasis that catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and phosphate in the terminal step of gluconeogenesis and glycogenolysis [77]. Glycogen storage disease type Ib (GSD-Ib) is caused by the mutation in *SLC37A4* gene (11q23.3) resulting in the deficiency of microsomal glucose-6-phosphatase transporter (G6PT). G6PT and G6Pase-alpha work in concert to maintain glucose homeostasis. Whereas G6Pase is exclusively expressed in gluconeogenic cells, G6PT is ubiquitously expressed and its deficiency generally causes a more severe phenotype.

Patients with GSD-Ia manifest a phenotype of disturbed glucose homeostasis characterized by life-threatening hypoglycemia, hepatomegaly, nephromegaly, hypercholesterolemia, hypertriglyceridemia, hyperuricemia, lactic acidemia, and growth retardation. Infants with GSD-Ib typically present with seizures and hepatomegaly around 6–8 months of age. Approximately 75% of adolescents and adults develop liver adenoma [78].

Renal symptoms and complications include nephromegaly, gout nephropathy, renal stones, nephrocalcinosis, FS, and chronic kidney disease [79]. Hyperuricemia and uric acid stones are caused by a combination of increased synthesis of purine and competitive inhibition of renal tubular excretion of uric acid by lactate [80]. Patients develop proximal renal tubular acidosis, hyperphosphaturia, generalized aminoaciduria, and increased excretion of LMW proteins. Renal biopsies in patients show interstitial fibrosis, tubular atrophy, and focal segmental glomerulosclerosis, with glomerular enlargement and thickening and lamellation of the glomerular basement membrane (GBM) [82]. Glycogen depositions are present in both tubular and glomerular cells.

Diagnosis is made by molecular genetic testing of *G6PC* and *SLC37A4*. Genetic testing has largely replaced traditional liver biopsy to measure enzyme activity (G6Pase catalytic activity and G6P exchanger SLC37A4 activity). Nonetheless, liver biopsy may still be useful in selected cases to investigate liver pathology and to test variants of uncertain significance.

Treatment is aimed at maintaining normoglycemia to avoid metabolic complications and lactic acidosis. Small frequent meals and snacks with high complex carbohydrate content are the mainstay of treatment [83]. During infancy, normoglycemia is accomplished at night with nasogastric tube feeding of glucose or with orally administered uncooked cornstarch. Recommended uncooked cornstarch doses are 1.6 g/kg body weight every 4 h for infants, 1.7–2.5 g/kg body weight every 6 h for young children through puberty, and 1.7–2.5 g/kg body weight given before bedtime for adults [84].

Progression of renal disease is markedly improved by strict adherence to diet [80, 81, 148, 149]. Tubular function abnormalities can also partially recover in very compliant patients [81]. ACE inhibitors have a demonstrated renoprotective effect in GSD-Ia [149, 150] and lipid-lowering agents may be beneficial. However, despite optimal treatment, glomerular hyperfiltration develops over time in most patients, and some progress to end-stage kidney disease [83, 148]. Liver transplantation is indicated in patients when medical treatment fails to achieve metabolic control, or when liver adenoma or hepatocellular carcinoma develop. Isolated or combined kidney and/or liver transplantation have been successfully performed [151, 152]. Living-donor liver transplantation is a viable option to restore normal metabolic balance and preserve kidney function [85, 152]. Hepatocyte transplantation is also used to prevent hypoglycemia and allow discontinuation of cornstarch meals [154].

Fanconi–Bickel Syndrome

Fanconi–Bickel syndrome (FBS) is an autosomal recessive disorder of monosaccharide transport, usually presenting in the first year of life with characteristic "doll-like" facies, hepatomegaly, nephromegaly, and severe FS causing rickets [86]. Patients with FBS accumulate glycogen in hepatocytes and proximal tubule cells, and develop hypoglycemia and galactose intolerance [89]. Some are diagnosed with early onset cataract [87]. The overall prognosis of FBS is usually favorable, but cases of fatal liver failure during infancy have been reported [88, 89].

The disease is caused by variants in the *SLC2A2* gene (3q26.2), encoding the GLUT2 facilitative glucose transporter, which is expressed in hepatocytes, pancreatic beta cells, and in the basolateral membranes of intestinal and renal tubular epithelial cells [156]. The inability of the liver to transport glucose, together with heavy renal losses of glucose, cause preprandial hypoglycemia [89]. Rarely, decreased monosaccharide uptake by the liver can cause postprandial hyperglycemia and hypergalactosemia, which is exacerbated by inappropriately low insulin secretion due to abnormal glucose-sensing by pancreatic beta cells. Hyperglycemia and glycosuria are sometimes the first signs of the disease in the neonatal period [158]. Renal glomerular hyperfiltration, microalbuminuria, and diffuse mesangial expansion have been reported in one child [90].

Disease severity is variable with no genotype phenotype correlation [156]. Some patients are misdiagnosed as having autosomal recessive or idiopathic FS. Compared to other forms of FS, patients with FBS have particularly severe glucosuria and galactosuria. The diagnosis is confirmed by molecular genetic analysis of *SLC2A2*.

Treatment is symptomatic and includes frequent meals and management of the FS [89]. This usually requires vitamin D3 and oral phosphate, and, depending on to the severity of the proximal tubulopathy, alkali, and salt supplementation. Frequent feeds, including at night, help prevent hypoglycemia and ketosis. Uncooked cornstarch and nocturnal enteral nutrition have been shown to decrease the frequency of hypoglycemic episodes and improve growth [91, 157]. Patients should eat fructose as their main source of carbohydrates and should avoid galactose to limit accumulation of glycogen in the liver and in kidneys.

Tyrosinemia Type I

Hereditary tyrosinemia type I (HTI) is an autosomal recessive disorder characterized by elevated blood levels of tyrosine. HTI is caused by mutations in the *FAH* gene (15q25.1), which encodes for the fumarylacetoacetate hydrolase (FAH) enzyme that converts fumarylacetoacetate into acetoacetate and fumarate [159, 160]. If mutated, L-tyrosine metabolism is impaired, and fumarylacetoacetate accumulates and is converted primarily into succinylacetone (Fig. 4) [159]. Succinylacetone is a toxic metabolite that causes FS by inhibiting brush border transporters, altering plasma membrane fluidity, and impairing oxygen consumption [163]. Inactivation of the L-tyrosine pathway upstream of the fumarylacetoacetate hydrolase reaction causes accumulation of tyrosine without significant renal disease [164], whereas direct

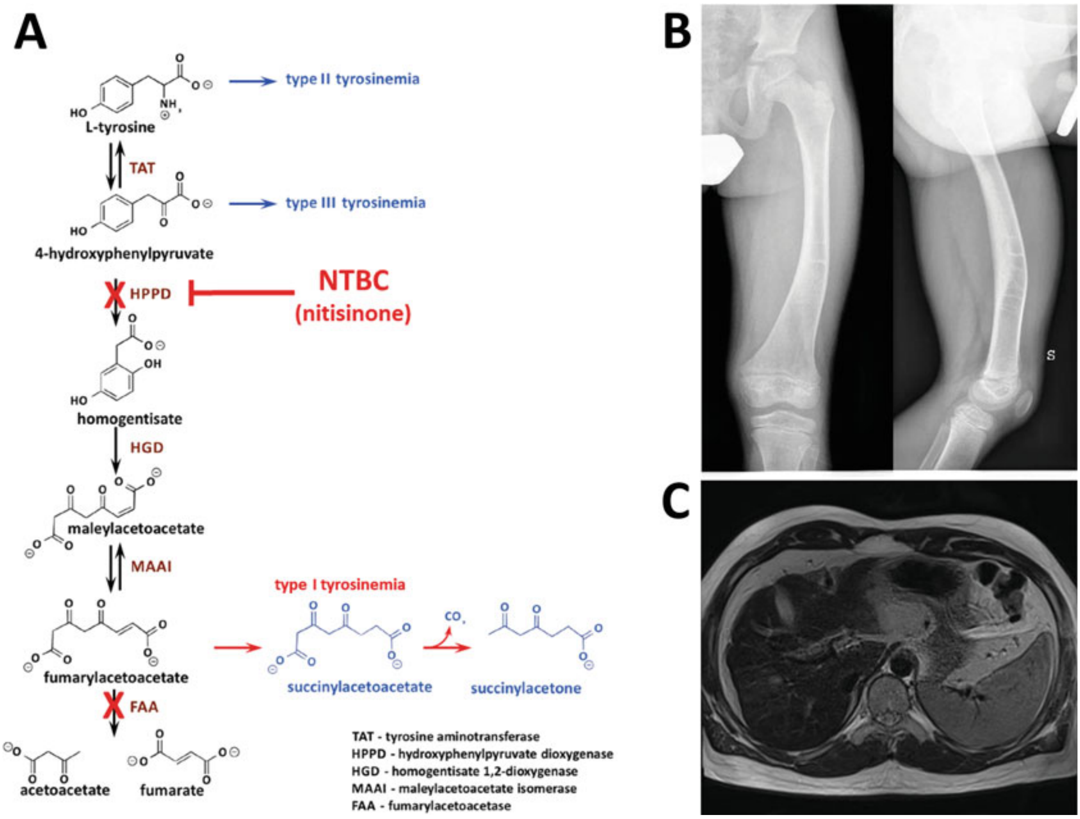

A
L-tyrosine
type II tyrosinemia
TAT
4-hydroxyphenylpyruvate
type III tyrosinemia
HPPD
NTBC
(nitisinone)
homogentisate
HGD
maleylacetoacetate
MAAI
fumarylacetoacetate
type I tyrosinemia
succinylacetoacetate
CO2
succinylacetone
FAA
acetoacetate
fumarate
TAT - tyrosine aminotransferase
HPPD - hydroxyphenylpyruvate dioxygenase
HGD - homogentisate 1,2-dioxygenase
MAAI - maleylacetoacetate isomerase
FAA - fumarylacetoacetase
B
S
C

Fig. 4 Panel A illustrates the metabolic pathway of tyrosine metabolism. Tyrosinemia type I is caused by pathogenic variants in the FAH gene that encodes the fumarylacetoacetase enzyme (FAA). Loss of function of FAA leads to accumulation of succinylacetone that causes proximal tubular dysfunction. Nitisinone (NTBC) inhibits the tyrosine pathway upstream of the FAA reaction, preventing synthesis of succinylacetone. Panel B shows severe rickets in a 3-year-old patient with tyrosinemia type I. Panel C shows the abdominal CT-scan of the same patient, demonstrating diffused nodular changes of the liver parenchyma [161]

injection of succinylacetone in rats causes proximal tubular damage [165]. In humans, the FS improves after succinylacetone levels decrease following liver transplantation [95, 165].

Most patients present in early infancy with acute liver failure [160]. A more subacute clinical presentation occurs between the ages of 6 and 24 months, and is characterized by failure to thrive, hepatosplenomegaly, coagulopathy, FS, and progressive neurological symptoms [160]. These include painful peripheral neuropathy that can lead to transient paralysis, and autonomic dysfunction that is often complicated with hypertension and tachycardia. Rarely, patients develop a more chronic form of the disease and are diagnosed after the age of 2 years with subclinical hepatic and renal tubular dysfunction [160]. Patients with HTI are at high risk of developing hepatocellular carcinoma [92]. In addition to tyrosine, they have elevated plasma concentrations of methionine, phenylalanine, and aminolevulinic acid, and excrete high concentrations of tyrosine metabolites in their urine [160]. Liver function tests are usually normal or moderately elevated. Alpha-fetoprotein is markedly increased, in particular in patients with hepatocellular carcinoma.

The FS of HTI is typically associated with hypophosphatemic rickets (Fig. 4). Generalized aminoaciduria, renal tubular acidosis, and LMW proteinuria are often present, whereas glucosuria is less common. Kidneys are often enlarged, and nephrocalcinosis is occasionally observed.

Glomerulosclerosis and chronic kidney disease develop progressively in untreated patients (Fig. 5).

The diagnosis of HTI is usually made by newborn screening, using tandem mass spectrometry to detect succinylacetone [93, 160], and is confirmed by molecular testing. Treatment should be started as soon as possible. It includes dietary restriction of tyrosine and phenylalanine, along with nitisinone (2-[2-nitro-4-trifluoromethylbenzoyl]-1,3-cyclohexanedione – NTBC) to block the tyrosine pathway upstream of the fumarylacetoacetate hydrolase reaction, preventing accumulation of succinylacetone and other potentially toxic metabolites [94]. FS improves significantly with NTBC treatment [161], and after liver transplantation [95, 165]. However, in some patients, liver transplantation alone cannot abolish urinary succinylacetone excretion and renal disease may still progress [165].

Wilson Disease

Wilson disease is an autosomal recessive inborn error of copper (Cu) metabolism with an estimated frequency of 1:30,000 [166]. The disease is caused by pathogenic variants in the *ATP7B* gene (13q14.3) that encodes for the Cu-transporting ATPase 2 [96].

Copper is present in excess in the diet; therefore, its levels in the body are primarily regulated by biliary excretion. Copper homeostasis in cells and tissues is maintained by a number of proteins including transporters, storage molecules (metallothioneins), cytosolic carriers, and enzymes that use Cu as a co-factor [166]. After intestinal absorption, Cu is released into the circulation and reaches the liver tagged with albumin. In the liver, Cu-transporting ATPase 2 promotes biliary Cu excretion (Fig. 5). In addition, it mediates

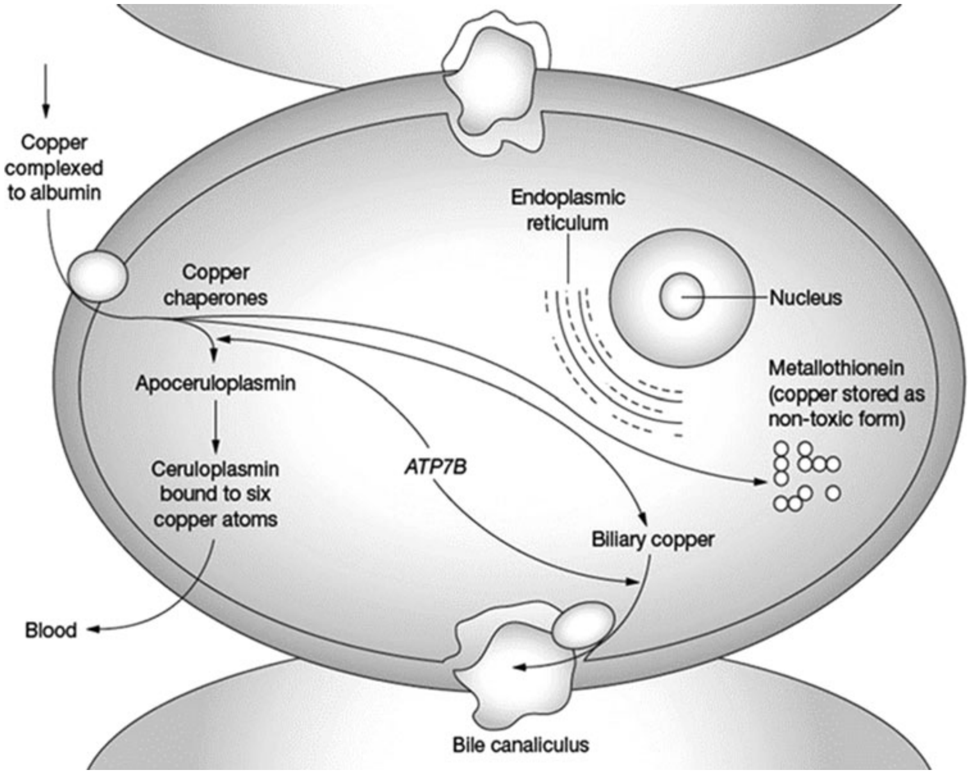

Copper complexed to albumin
Endoplasmic reticulum
Copper chaperones
Nucleus
Apoceruloplasmin
Metallothionein (copper stored as non-toxic form)
Ceruloplasmin bound to six copper atoms
ATP7B
Biliary copper
Blood
Bile canaliculus

Fig. 5 Schematic representation of copper metabolism within a liver cell. *ATP7b*, causative gene for Wilson disease [96]

incorporation of Cu into apoceruloplasmin, the precursor of ceruloplasmin. Ceruloplasmin carries 90% of circulating Cu and transfers Cu to peripheral tissues [167]. Mutations in the *ATP7B* gene cause decreased conversion of apoceruloplasmin into ceruloplasmin, which is often low in patients with Wilson disease. As a result, Cu circulates loosely bound to albumin and other proteins, and is continuously up taken by tissues. Most clinical symptoms of Wilson disease are directly related to Cu toxicity. Decreased biliary excretion further increases Cu accumulation and toxicity in hepatocytes. Copper excess induces oxidative stress that causes damage to lipids, proteins, and nucleic acids [166]. It also induces apoptosis and inhibits the activity of several enzymes [166]. Mitochondria, in which PT cells are particularly reach, are very sensitive to Cu-induced toxicity [166]. More than 700 different pathogenic variants in *ATP7B* have been reported. The R778L variant is very frequent in south-east Asia, the H1069Q variant is predominant in patients of central, eastern, and norther European ancestry, while other genetic changes are typical in southern Europe [166]. Phenotype-genotype correlations have been evaluated in numerous studies with no consistent findings.

Patients with Wilson disease develop neurological and liver lesions over time. Symptoms rarely manifest before 6 years of age. Hepatic signs are present at onset in 40–60% of subjects. Most frequently, patients develop progressive hepatic steatosis, which can be diagnosed by ultrasonography or liver biopsy. Liver function tests are mildly elevated and, if left untreated, liver disease progresses to cirrhosis [166]. In other cases, patients present with acute liver failure, in particular young females [166]. Neurological symptoms are the second most frequent presenting symptoms and are reported in 20–60% of patients [167]. They are heterogeneous, but include movement disorders, such as tremors, involuntary movements, dystonia, dysarthria, Parkinsonism, or dysphagia; less frequently, patients develop seizures [97]. Approximately 20% of patients have neuropsychiatric manifestations at diagnosis. Other presenting features include hemolytic anemia, bone fractures, arrhythmias, FS, skin hyperpigmentation, sunflower cataract, and the characteristic Kayser-Fleischer ring. This latter feature is caused by Cu accumulation in the cornea, and is observed in 20–30% of asymptomatic patients, 40–50% of patients with liver disease, and nearly all patients with overt neurological symptoms [166]. Menstrual irregularities, delayed puberty, gynecomastia, and miscarriage are also frequent.

In the kidney, accumulation of Cu causes tubular dysfunction. Patients can develop FS before overt hepatic disease, including intermittent glucosuria, aminoaciduria, hyperphosphaturia, hyperuricosuria, or LMW proteinuria [98]. Other urinary findings include hypercalciuria, urolithiasis, nephrocalcinosis, decreased urine concentrating ability, and distal renal tubular acidosis. Kidney function progressively deteriorates in untreated patients, but usually death occurs from nonrenal causes before the onset of end-stage kidney disease.

The diagnosis of WS is based on a combination of clinical and laboratory signs because none of the available laboratory tests has sufficient specificity and sensitivity. A score that integrates clinical and laboratory findings has been developed, and has been shown to have good diagnostic accuracy. Serum ceruloplasmin is usually low, but may be normal in patients with active liver disease. The 24-h urinary Cu excretion is increased in 80% of patients, but may be normal, in particular in children without liver disease and in asymptomatic subjects. Non-ceruloplasmin-bound Cu is usually increased, but may be normal, in particular if ceruloplasmin is measured by immunoassay. Liver Cu is increased, but requires a liver biopsy and is not homogeneous across the hepatic tissue, in particular if patients have active disease and develop regenerative nodules. The Kayser-Fleischer ring is absent in most asymptomatic patients and in 50% of patients with hepatic WD. Genetic analysis of the *ATP7B* gene gives the diagnosis. Next-generation sequencing techniques have largely replaced biochemical tests. Molecular genetic screening should always be performed in asymptomatic siblings.

Treatment options include pharmacological therapy and liver transplantation. Medical treatment is lifelong and should not be discontinued, unless liver transplantation is performed.

Pharmacological therapy aims at achieving a negative Cu balance and preventing Cu re-accumulation [99]. Patients should be instructed to avoid Cu-rich foods including chocolate, nuts, shellfish, mushrooms, and liver, especially during the first year of treatment.

Chelating agents include D-penicillamine and trientine. D-penicillamine therapy has been the most commonly used chelating agent, and has been shown to reverse at least in part, most symptoms, including renal tubular dysfunction. Careful monitoring for side effects, including hypersensitivity reactions, proteinuria, Goodpasture's syndrome, polymyositis, systemic lupus erythematosus, skin changes, and bone marrow suppression, is essential. Trientine is another effective chelating agent and is often better tolerated than D-penicillamine. In addition to Cu, it also chelates iron, and should not be administered with iron supplements because it forms a toxic compound. Reversable sideroblastic anemia and lupus-like symptoms have been reported. Ammonium tetrathiomolybdate chelates Cu very efficiently, but at high doses can form an insoluble copper complex, which is deposited in the liver; it is not commercially available. Zinc salts interfere with the uptake of copper from the gastrointestinal tract and help reduce intestinal Cu absorption. They have few side effects. It may cause gastric irritation, mild immunosuppression, or pancreatic irritation. Successful therapy is assessed by monitoring serum non-ceruloplasmin-bound copper, and 24-h urinary Cu excretion. The 24-h urinary copper excretion on medication and after 2 days of cessation of therapy should be measured annually to assess the effectiveness of therapy and adherence to treatment. Treatment of presymptomatic patients and patients on maintenance therapy can be accomplished with a chelating agent or with zinc. During pregnancy, therapy should be decreased but not discontinued.

Patients with acute liver failure or decompensated cirrhosis, unresponsive to chelation treatment, should be offered liver transplantation; living-donor transplantation has been safely performed.

Lysinuric Protein Intolerance [100–102]

See ▶ Chap. 31, "Renal Manifestations of Metabolic Disorders in Children."

Microvillous Inclusion Disease

Microvillous inclusion disease (MVID) is a severe congenital intestinal malabsorption syndrome that presents with intractable secretory diarrhea within the first days or weeks of life. Patients are fully dependent on parenteral nutrition (TPN) for survival [103]. The disease is caused by biallelic mutations of the *MYO5B* gene (18q21.1) coding for myosin Vb, an actin-related motor protein that interact with endosomal Rab GTPases, allowing recycling to the plasma membrane [104]. Lack of expression of myosin Vb impairs apical brush border formation and cell polarity in enterocytes. FS has been reported in patients treated with TPN, but no clear ultrastructural defects of PT cells have been observed in kidney biopsies from these patients [105]. The diagnosis is made by intestinal biopsy and molecular genetic testing of *MYO5B*. Treatment is supportive and involves long-term maintenance of nutrition and hydration with TPN, and consideration of intestinal transplantation.

Maturity-Onset Diabetes of the Young (MODY)

Maturity-onset diabetes of the young (MODY) is characterized by autosomal dominant inheritance, onset before 25 years of age, absence of β-cell autoimmunity, and sustained pancreatic β-cell function. To date, pathogenic variants have been identified in at least 14 different genes [106]. MODY is a rare condition, accounting for 1–6% of pediatric cases of diabetes mellitus. Two subtypes (MODY1 and MODY5) show renal manifestations.

MODY1 is caused by variants in *HNF4A* (20q13.12), which encodes the transcription factor HNF4α. HNF4α is expressed in liver, pancreas, and kidney. Phenotypically, MODY1 manifests progressive insulin deficiency. Fetal *HNF4α* heterozygosity results in macrosomia due to hyperinsulinemia in utero and subsequent neonatal transient or persistent hypoglycemia, which is responsive to diazoxide. Hyperinsulinism remits during infancy, followed by a gradual decrease in endogenous insulin production and diabetes mellitus in adolescence [107]. Patients with the specific R76W variant manifest autosomal dominant FS in addition to neonatal hyperinsulinism, young onset diabetes mellitus, and macrosomia [108]. Nephrocalcinosis, renal impairment, hypercalciuria with relative hypocalcemia, and hypermagnesemia are also seen.

MODY5 is caused by variants in *HNF1B* (17q12) which encodes the transcription factor HNF1β [109]. HNF1β regulates organogenesis of the kidney, genitourinary tract, liver, lungs, gut, and pancreas. Renal malformations, including cysts, dysplasia, urinary tract malformation including hydronephrosis, hydroureter and vesicoureteral reflux, hypoplastic kidney disease, and rarely FS, are associated [110]. Carriers of *HNF1B* mutations can have significantly reduced birth weight. Patients manifest hypokalemia or hypomagnesemia in 60% of cases. They typically develop CKD in the fifth decade of life, and half of patients will progress to end-stage kidney disease without developing diabetic kidney disease. Genitourinary tract malformations (especially uterine abnormalities), liver dysfunction, and pancreatic hypoplasia have also been reported. Patients with MODY5 present with dyslipidemia, including a low high-density lipoprotein level and elevated triglyceride level.

Type IIa Sodium/Phosphate Cotransporter (NaPi-IIa) Deficiency

Mutations in the *SLC34A1* gene (5q35.3), which encodes type IIa sodium/phosphate cotransporter (NaPi-IIa), cause idiopathic infantile hypercalcemia with hypercalciuria and nephrocalcinosis, also termed infantile hypercalcemia type 2 [112] (see ▶ Chap. 40, "Renal Hypophosphatemia").

Two siblings with additional features of incomplete FS, including glucosuria, aminoaciduria, hyperuricosuria, and LMW proteinuria have been described. They carried a homozygous in-frame duplication of 21 bp in the *SLC34A1* gene. Unlike previously described *SLC34A1* mutations, both patients had normal serum calcium, no hypercalciuria and nephrocalcinosis, and moderate Vit D deficiency [111].

Arthrogryposis-Renal Dysfunction-Cholestasis (ARC) Syndrome

Arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome is a fatal autosomal recessive multisystem disorder caused by variants in the *VPS33B* (15q26.1; homolog of the class C yeast vacuolar sorting protein) or *VIPAR* (14q24.3; VPS33B interacting protein, apical-basolateral polarity regulator, spe-39 homolog) genes. VPS33B and VIPAR are essential for lamellar body biogenesis and function [113]. The classical presentation of ARC syndrome includes congenital joint contractures, renal tubular dysfunction, and cholestasis. Additional features include ichthyosis, central nervous system malformation, platelet anomalies, and growth faltering. Diagnosis of ARC syndrome is based on clinical features with molecular genetic confirmation [113]. Patients manifests FS with glucosuria, aminoaciduria, phosphaturia, acidosis, and LMW proteinuria. In addition, they can have nephrogenic diabetes insipidus. Nephrocalcinosis, renal dysplasia, glomerular sclerosis, and tubular degeneration are reported [114]. There is no specific treatment and clinical management is supportive.

Idiopathic Fanconi Syndrome

Idiopathic FS is a diagnosis of exclusion, when no underlying cause can be identified. Whole exome sequencing of index cases and family members can help identifying a genetic cause.

Acquired Forms of Fanconi Syndrome

Endogenous Causes

Rarely, patients with nephrotic syndrome develop FS. Kidney histology usually shows focal segmental glomerulosclerosis, with or without tubulointerstitial changes [115, 119, 167]. Similarly, patients with autoimmune interstitial nephritis, including TINU syndrome (acute tubulointerstitial nephritis with uveitis), occasionally develop FS [119]. Corticosteroid therapy in these patients may improve tubular dysfunction.

Several other immunological or hematological disorders can be associated with FS, mostly in adults. These include Sjögren syndrome, multiple myeloma, monoclonal gammopathy, and lymphoma.

Sjögren syndrome is an autoimmune connective tissue disorder that affects exocrine glands [116]. Renal involvement of Sjögren syndrome is mainly manifests as tubular dysfunction. Seventy percentage patients manifest distal renal tubular acidosis [116]. Urinary concentration defects and LMW proteinuria are also often observed. Less than 5% of patients with Sjögren syndrome develop FS; they usually suffer osteomalacia, cortical bone thinning, and multiple fractures. Kidney histology shows chronic interstitial nephritis, with diffuse or focal lymphocytic infiltration. Treatment includes corticosteroid and immunosuppressant therapy.

FS is a typical finding in adults with myeloma, who have crystalline deposition of light chains in the cytoplasm of PT cells; the disease often progresses to severe tubulointerstitial nephritis and end-stage kidney disease.

Adult patients with paroxysmal nocturnal hemoglobinuria (PNH) can also manifest FS as a result of chronic hemoglobinuria and hemosiderin deposition in PT cells. By preventing intravascular hemolysis, anti-C5 therapy with eculizumab [117] significantly decreases the iron load to the kidney and the risk of developing FS.

Untreated children with distal renal tubular acidosis may present with features of FS, including LMW proteinuria, aminoaciduria and, less commonly, phosphaturia [122, 169]. Glycosuria is usually absent [169]. In one report, proximal tubular dysfunction was noted in 67% of patients with distal renal tubular acidosis at presentation [169]. The proximal tubulopathy is probably secondary to acidosis, as it resolves with treatment. A similar mechanism may explain cases of FS in association with anorexia nervosa, which improves with nutritional support [121].

Very rare cases of acquired FS have been reported after Legionella pneumonia infection [120].

Exogenous Causes

Drugs

A variety of drugs and herbs can cause FS (Table 2) [123]. The incidence of PT toxicity from these compounds is almost certainly underestimated. Most of these compounds are filtered by the glomeruli and reabsorbed in proximal tubular cells, where they accumulate at high concentrations, explaining the predominant toxicity of these drugs to this segment of the nephron. Various mechanisms are responsible for tubular epithelial cell toxicity, including direct damage of the brush border, inhibition of mRNA synthesis, mitochondrial toxicity, oxidative stress, abnormal lysosomal function, inhibition of endocytosis and intracellular trafficking, and altered phospholipid metabolism. The list of culprits is vast and includes anticancer agents, tetracyclines, aminoglycosides, rifampicin, salicylate [124], suramin, fumaric acid, valproic acid [125], and Chinese herbs [126]. These herbs contain aristolochic acids that cause PT injury, and the phenomenon is often referred to as “aristolochic acid-related nephropathy.”

A number of cancer chemotherapy agents cause FS. Their nephrotoxic effects are dose dependent and often irreversible. Ifosfamide (IFO) is an alkylating agent widely used in the treatment of solid tumors [127]. Patients treated with both IFO and cisplatin have a high risk of developing FS. Chloroacetaldehyde (CAA), one of the main metabolites of IFO, is probably responsible for its nephrotoxicity. CAA alters mitochondrial respiration, increases oxidative stress, decreases glutathione and ATP levels, inhibits intracellular trafficking, and alters expression of several genes [128, 129]. Electron microscopy performed on kidney biopsies of patients with IFO toxicity often shows irregular and enlarged mitochondria in PT cells. Ultrasound

Table 2 Causes of drug-induced renal FS. (Partly modified from Ref. [123]

Class	Drugs	Indications for use
Alkylating agents	Ifosfamide	Cancer
Aminoglycoside antibiotics	Gentamicin, amikacin	Gram-negative bacterial infection
Antiepileptics	Sodium valproate	Seizures, bipolar disorder
Anti-protozoals	Suramin	Trypanosomiasis
Chinese herbs	Aristolochic acid	Edema, health promotion
Dicarboxylic acids	Fumaric acid	Psoriasis
Iron chelators	Deferasirox	Iron overload
Nucleotide analogue reverse transcriptase inhibitors (NRTIs)	Didanosine, stavudine	HIV
Nucleotide reverse transcriptase inhibitors (NtRTIs)	Adefovir, cidofovir, tenofovir	HIV, hepatitis B, CMV
Platinum compounds	Cisplatin, carboplatin	Cancer
Salicylates	Aspirin	Analgesia, anti-inflammatory
Tetracycline antibiotics	Degraded tetracycline	Bacterial infection
Tyrosine kinase inhibitors	Imatinib mesylate	Chronic myeloid leukemia

usually shows hyperechogenicity, with preserved corticomedullary differentiation. A number of compounds, including L-carnitine, taurine, or N-acetyl cysteine, have been proposed to reduce IFO-induced nephrotoxicity [130], but to date, none have proved effective in the clinical practice.

Cisplatin enters PT cells through basolateral organic anion transporters and induces the formation of reactive thiols and monohydroxy complexes that are highly toxic to the PT cell [131]. In addition, it stimulates proapoptotic and inflammatory pathways, and inhibit several transporters [131].

Imatinib mesylate is a specific tyrosine kinase inhibitor that is used as first-line therapy for patients with chronic myeloid leukemia. This agent can induce partial FS including phosphaturia and uricosuria with AKI. Combined blockade of both platelet-derived growth factor receptor and c-Kit receptor tyrosine kinase in proximal tubules can also cause partial FS [132].

Nucleotide reverse transcriptase inhibitors that are used as anti-human immunodeficiency virus (HIV) agents including didanosine, stavudine, adefovir, cidofovir, and tenofovir can induce FS, nephrogenic diabetes insipidus, and acute renal failure [133–135]. Adefovir is also used for the treatment of chronic hepatitis B infection. Adefovir and cidofovir enter PT cells by basolateral organic anions transporters and efflux into the tubular lumen through the multidrug-resistance protein 2 (MRP2) located in the luminal membrane. If efflux is inhibited, these drugs accumulate in the proximal tubule cells, leading to mitochondrial damage and tubular toxicity. The cytotoxicity of adefovir and cidofovir is proportional to the expression of basolateral organic anions transporters (OAT). Histological and ultrastructural examination reveals degenerative changes of proximal tubules with swollen and dysmorphic mitochondria. Tenofovir is relatively less nephrotoxic, probably because it interacts less with OAT.

Nephrotoxicity can also result from crystallization of drugs within proximal tubules. Crystalline-induced tubular injury is exacerbated by low urinary flow rate and high concentration of the compound in the tubular lumen, when administered at high concentrations or with fast infusion rates. Examples include methotrexate, indinavir, acyclovir, atazanavir, sulfadiazine, ciprofloxacin, or triamterene.

Deferasirox, which prevents hemosiderin deposition in proximal tubules, rarely can cause reversible FS [136].

Other Chemical Compounds

A large number of chemical compounds exhibit PT toxicity. Paraquat, a nonselective herbicide, and colloidal bismuth subcitrate, both cause FS

[137, 138]. Large amounts of these compounds can be ingested in suicide attempts. Patients develop FS and AKI. Treatment with the chelating agent sodium-2,3-dimercapto-1-propanesulfonate in combination with hemodialysis is highly effective in reducing serum bismuth levels. Similarly, intoxication with other compounds, such as methyl-3-chromone (diachrome), 6-mercaptopurine, and toluene, can cause FS.

Heavy Metals

Heavy metals including lead, cadmium, mercury, chromium, and platinum are major environmental and occupational hazards. They are toxic at low doses, and induce mitochondrial toxicity and cell oxidative damage. The kidney is often the first organ affected. The extent of renal damage depends on the dose, route of intoxication, and duration of exposure. Both acute and chronic intoxications have been reported, with varying degrees of severity, ranging from subclinical tubular dysfunction to severe AKI [139]. Lead poisoning often leads to FS, in particular in children [140]. Since lead has a very long half-life, tubular dysfunction can persist for more than 10 years after severe poisoning [141].

Similarly, prolonged exposure to cadmium can cause FS [142]. Cadmium contained in the industrial waste in the Jinzu River basin in Toyama (Japan) was responsible for numerous cases of "*Itai-Itai*" (*ouch-ouch*) disease, which corresponds to FS associated with severe osteomalacia and frequent spontaneous fractures [143].

Therapy

Identification of the underlying cause of FS is the first step in establishing appropriate therapy. Avoidance of offending nutrients in galactosemia, HFI, and tyrosinemia, and avoidance of Cu-rich foods in Wilson disease are critical. Specific treatments with Cu-chelating agents detailed above can be effective for Wilson disease. Immunosuppressive drugs are used for immunological diseases, including Sjögren syndrome, TINU syndrome, or autoimmune interstitial nephritis. Exogenous substances should be stopped or reduced in dose if complete avoidance is not possible. Normalization of tubular function can occur after these treatments and withdrawal of toxic agents, but complete recovery of FS can take several months.

When a specific treatment is not available, therapy is aimed at ameliorating biochemical abnormalities secondary to renal solute and fluid losses and the metabolic bone disease. Proximal renal tubular acidosis usually requires large amount of alkali (2–15 mEq/kg of body weight per day) divided into four to six daily doses. Excessive alkali substitution may cause volume expansion, thereby exacerbating bicarbonate wasting. Potassium citrate, potassium bicarbonate, or potassium acetate treat both acidosis and hypokalemia simultaneously. Sodium wasting and dehydration are treated with combination of sodium bicarbonate, citrate, and chloride, depending on the degree of acidosis. Ensuring adequate fluid and electrolyte intake is essential, especially in the case of infants or gastrointestinal disease. Early intervention with intravenous replacement therapy may be required for patients with vomiting and diarrhea.

Hypophosphatemia and impaired renal vitamin D_3 metabolism can lead to rickets. This is treated with phosphate supplements and $1,25(OH)_2D_3$ or dihydrotachysterol, avoiding hypercalcemia and hypercalciuria, with may exacerbate nephrocalcinosis. Adequate physical activity, appropriate diet with calcium, phosphate, and vitamin D3 are recommended [144]. Growth faltering can be a major complication of FS. Despite treatment of electrolyte abnormalities, some patients manifest severe growth faltering, especially those with cystinosis and FBS. Growth hormone deficiency has also been reported in FS [145]. Treatment with growth hormone can improve growth in nephropathic cystinosis (see ▶ Chap. 37, "Cystinosis") and other selected cases.

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Cystinosis

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Abstract

Cystinosis is an autosomal recessive disorder caused by mutations in the *CTNS* gene that encodes for cystinosin, a lysosomal cystine/H^+ symporter. The disease causes progressive accumulation of cystine in virtually all tissues. In the most frequent and severe form, termed nephropathic infantile cystinosis, kidney involvement is responsible for the initial symptoms, with patients presenting in the first year of life with symptoms related to renal Fanconi syndrome. Cystine corneal crystals are present in all patients by the age of 2 years. The diagnosis is based on the detection of increased leucocyte cystine levels and the demonstration of mutations in the *CTNS* gene. Cysteamine was introduced in the 1980s for the treatment of cystinosis. Cysteamine has considerably improved the prognosis of the disease and delays progression to end-stage kidney disease by approximately 10 years. If not appropriately treated, patients rapidly develop other symptoms, including retinal degeneration, hypothyroidism, diabetes mellitus, exocrine pancreatic insufficiency, pubertal retardation, gonadal dysfunction, restrictive pulmonary disease, myopathy, neurological deterioration, and liver involvement. Two milder forms of the disease, namely juvenile cystinosis and ocular cystinosis, have also been described. These patients present with milder symptoms later in childhood or with isolated corneal cystine depositions, respectively.

Keywords

Cystinosis · *CTNS* gene · Renal Fanconi syndrome · Cysteamine

Introduction

Overview

Nephropathic cystinosis deserves a special place in the annals of clinical medicine as the first treatable lysosomal storage disease. The pathophysiology itself, based upon the formation of cystine crystals within the lysosomes of cells, is remarkable. The presence of cystine crystals provides a clue to the basic defect in cystinosis, i.e., failure to transport cystine out of lysosomes. This created a new area of biomedical investigation, explained the lysosome's function in salvaging small molecules for reutilization by the cell, and revealed a new category of lysosomal storage disorders due to transport defects rather than enzyme deficiencies. Even more striking, a rational therapy of cystine depletion (i.e., cysteamine) emerged, transforming nephropathic cystinosis from a universally fatal disease to a treatable chronic disorder with a decent quality of life and increased life span. Today, physicians can even observe the gradual dissolution of cystine crystals by cysteamine eyedrops bathing the corneas of patients' eyes.

History

Cystinosis was first described by Abderhalden in 1903, when understanding of its renal disease remained rudimentary. Fanconi, de Toni, and Debré recognized the renal tubular defect of cystinosis in the 1930s, and this complication retains the appellation renal Fanconi syndrome today. Generalized aminoaciduria was noted as a concomitant of nephropathic cystinosis in the late 1940s, and cystine storage within cellular lysosomes was proven in the late 1960s. By 1982, the basic defect of impaired lysosomal membrane transport of cystine was reported, and therapy with the cystine-depleting aminothiol cysteamine was shown to be safe and effective by 1987. Since then, numerous extrarenal complications of cystinosis have been described, and oral cysteamine therapy has been shown to delay or prevent virtually all of them.

The Basic Defect

Cystine has a molecular weight of 240 Daltons (Da) and consists of two molecules of cysteine joined by a disulfide bond. The equilibrium between cystine and cysteine depends upon their redox potentials and the pH of the milieu; if a high enough concentration of cystine is present (>2 mM), it may precipitate out of solution because of its poor solubility. Cysteine, which is very soluble, is produced by the hydrolysis of proteins; this occurs within lysosomes by the action of acidic hydrolases. Cysteine is then oxidized to cystine within lysosomes, where it accumulates if the cell has the genetic defect of cystinosis [1, 2]. Cysteine can also be transported from the cytosole to the lysosomal lumen by MFSD12 transporter on the lysosomal membrane [3]. After decades of study, scientists understood that cystine could not exit the lysosomes of cystinotic cells. Indeed, normal polymorphonuclear leukocytes were able to clear their lysosomes of cystine, but cystinotic cells could not [4]. Studies of isolated granular fractions, i.e., lysosomes, gave similar results [5]. In fact, normal, cystine-loaded lysosomes could transport cystine in either direction across their membranes, while cystinotic lysosomes couldn't release cystine [6, 7]. Cystine transport in neutrophil lysosomes was subsequently shown to be ligand specific, stereospecific, and ATP dependent [8, 9]. It displayed classical saturation kinetics (Fig. 1) and counter transport. Similar findings were observed in cultured lymphoblasts [7, 9]. In total, these discoveries proved that the process of lysosomal cystine transport is carrier mediated and deficient in cystinosis. Indeed, heterozygotes for cystinosis displayed half the maximum velocity of cystine transport [10], consistent with having half the normal number of cystine carriers (Fig. 1). The later discovery of the cystinosis gene, *CTNS*, and studies of its gene product, cystinosin, demonstrated that this protein does indeed transport cystine, in a process driven by the hydrogen ion gradient across the lysosomal membrane [11, 12].

Cell damage in cystinosis is probably not related solely to lysosomal cystine accumulation. Recent studies have revealed cell abnormalities that are directly caused by defects in the cystinosin protein, independent of its transport activity [13, 14]. Supporting this view, not all aspects of the cell's cystinotic phenotype are corrected after lysosomal cystine depletion by cysteamine, which possibly explains why this drug, which has dramatically changed the outcome of cystinosis, cannot treat all symptoms completely. In particular, the Fanconi syndrome of cystinosis occurs in infancy, about the same time that renal tubules appear damaged early in

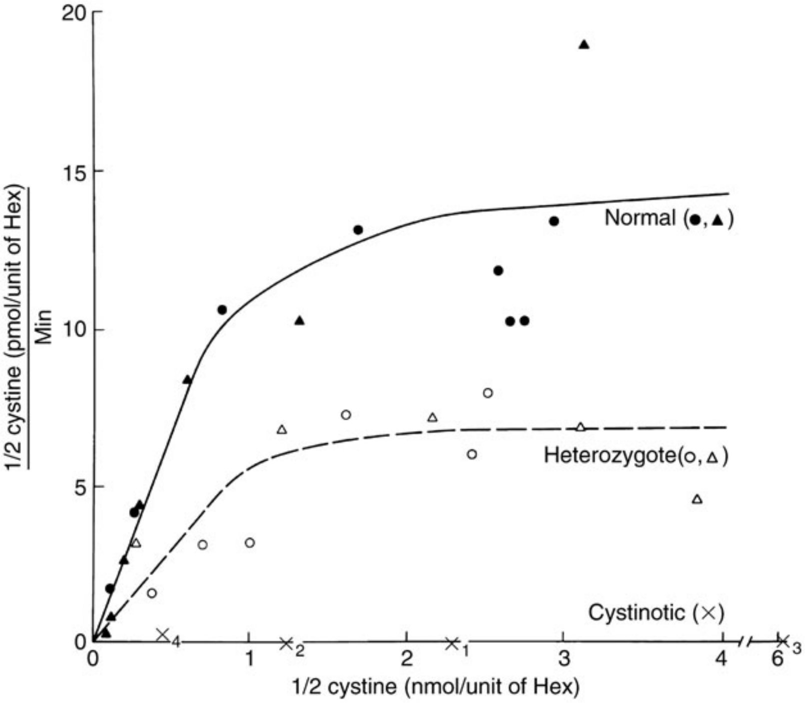


Fig. 1 *Lysosomal cystine transport in leukocyte granular fractions*. Normal lysosomes were loaded with cystine by exposure of whole leukocytes to cystine dimethylester, which is hydrolyzed to cystine within the acidic lysosome. Cystinotic lysosomes contain endogenously produced cystine. The abscissa gives the level of cystine loading per unit of hexosaminidase, i.e., per lysosome. The ordinate gives the rate of cystine egress in picomoles per minute per unit of hexosaminidase. Normal lysosomal cystine egress exhibits saturation kinetics, while cystinotic lysosomes show virtually no cystine egress. Heterozygotes for cystinosis, with half the number of lysosomal cystine transporters, display half the normal maximal velocity of cystine egress. (Reprinted from SCIENCE [5])

renal development [15]. Cells obtained from cystinotic patients or that have been generated in knockout experimental models are characterized by impaired endolysosomal dynamics and abnormal autophagy resulting in the accumulation of autophagosomes, mitophagosomes, and enlarged lysosomes around the perinuclear region. Studies have documented altered mTORC1 activity that is probably linked to altered interactions between cystinosin and proteins of the vacuolar ATPase, resulting in the activation of the transcription factor EB (TFEB). In proximal tubular cells, impaired endolysosomal trafficking compromises the recycling to the plasma membrane of the endocytic receptors megalin and cubilin, causing low-molecular weight proteinuria, as seen in Fanconi syndrome. Cell death may result from oxidative stress, mitochondrial damage, or direct stimulation of apoptosis. Impaired efflux of cystine from lysosomes has also been proposed to alter glutathione metabolism, exposing cells to oxidation. In proximal tubular cells, accumulation of large cystine crystals may rupture cell membranes directly, causing cell death. In addition, cystine crystals activate inflammatory pathways through NOD-like receptors. Despite these remarkable advances in the understanding of the physiopathology of the disease, it remains unclear which events are key elements causing cell dysfunction versus which represent downstream events.

Pathology

Patients who have not received long-term cystine-depleting therapy exhibit specific pathological features. As a consequence of excessive cellular cystine storage (to 10–1000 times normal levels), patients with cystinosis develop microscopic crystals of cystine, apparent within lysosomes on electron microscopy [2]. Tissues containing cystine crystals include the cornea, conjunctiva, iris, retina, liver, spleen, kidneys, intestines, rectal mucosa, pancreas, testes, lymph nodes, bone marrow, macrophages, thyroid, muscle, and choroid plexus [6, 16]. The crystals are generally hexagonal or rectangular and appear birefringent under polarizing light (Fig. 2). Tissue damage accompanies the intracellular cystine accumulation of cystinosis, with the greatest effects in the kidney. Renal tubules show a characteristic narrowing called a swan-neck deformity [15, 17], followed by interstitial nephritis and endothelial glomerular proliferation, necrosis, and hyalinization. Formation of atubular glomeruli and a glomerulo-tubular disconnect appears to represent a maladaptation to renal injury, contributing to the progression of renal insufficiency [17, 18]. Crystals are occasionally seen within glomeruli, which often contain large multinucleated podocytes [19]. Focal and segmental glomerulosclerosis (FSGS) lesions in cystinotic glomeruli can be undistinguishable from idiopathic FSGS. In the eye, the retina exhibits patchy hypopigmentation [20] and crystals appear occasionally in the iris and rarely in the retina [21] [22]. In older patients, the thyroid and testes appear fibrotic. Muscle histopathology involves a late vacuolar myopathy with variation in fiber size, atrophy of type I fibers, and ring fibers [23, 24]. In the liver of untreated adults, nodular regenerative hyperplasia can occur [25].

Genetics

Cystinosis is an autosomal recessive disorder, and heterozygotes never present clinical symptoms. The disease occurs with an incidence of approximately one in 100,000–200,000 live births, although there are genetic isolates of cystinosis, including one with a frequency of 1 in 26,000 in Brittany [26] and another among French Canadians. Within the US population, it is estimated that approximately one in 200 individuals carries a cystinosis (i.e., *CTNS*) mutation. A recent newborn screening program in Germany has identified 696 heterozygous carriers among 257,734 newborns, corresponding to a carrier frequency of one in 370 [27]. These numbers allow counseling of persons, known to be heterozygotes because a family member was diagnosed, with respect to their risk of having a child with cystinosis by an unrelated mate.

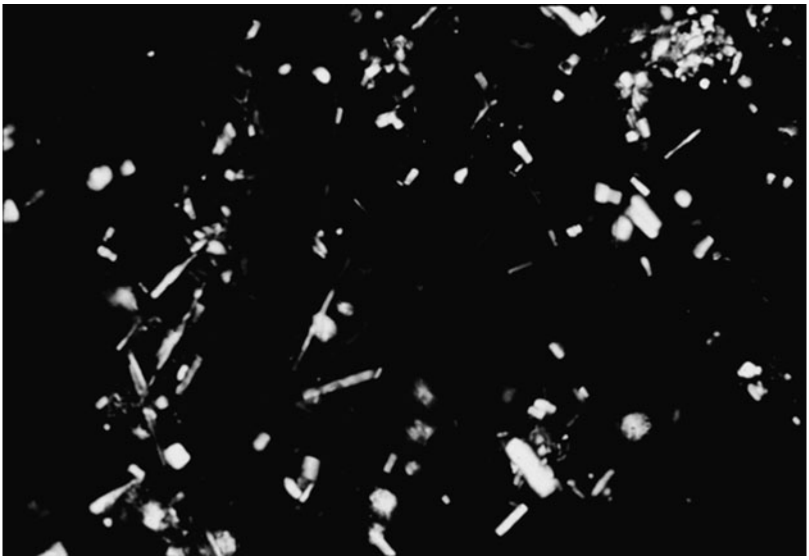


Fig. 2 *Birefringent cystine crystals in cystinosis tissue under light microscopy.* Note rectangular and needlelike shapes. Some crystals are undergoing dissolution due to the aqueous fixative

The *CTNS* Gene

The cystinosis gene was mapped to chromosome 17p13 in 1995 [28] and identified in 1998 [11]. It contains 12 exons within 23 kb of genomic DNA and codes for a 367-amino-acid protein, cystinosin, with seven transmembrane domains. The function of cystinosin as a cystine transporter has been demonstrated [12]. Over 160 different *CTNS* mutations, including deletions, insertions, nonsense, missense, and splice site mutations, have been reported in the Human Gene Mutation Database; they are found in different combinations in individuals with cystinosis [29–31]. The promoter [32], leader sequence, transmembrane regions, and non-transmembrane regions are affected by different mutations. The most common mutation is a 57,257-bp deletion removing the first 9 exons and part of exon 10 of *CTNS* [32, 33]. This deletion arose in Germany ~500 AD and is present in the homozygous or heterozygous state in more than half of cystinosis patients of European descent [33]. Homozygosity for this deletion might be associated with a slightly greater frequency of non-renal complications of cystinosis in adulthood and of enhanced intrinsic skin aging [31, 34]. Hotspot mutations other than 57-kb deletion are reported in different populations [31].

Clinical Aspects

Cystinosis Variants

Traditionally, cystinosis has been divided into three subtypes [6]. Infantile nephropathic cystinosis (OMIM 219800) is the classic disease, described below and comprising 95% of cases. Affected individuals have two severe *CTNS* mutations. Patients with intermediate (formerly termed “juvenile” or “adolescent” cystinosis; OMIM 219900) cystinosis have milder disease, with diagnosis in childhood, adolescence, or early adulthood; they eventually develop renal failure. Intermediate patients account for less than 5% of cases and generally carry one severe and one mild *CTNS* mutation [31, 35]. These patients usually present with asymptomatic proteinuria and mild or absent tubulopathy. Over time, they develop lesions that may be misdiagnosed as primary focal segmental glomerulosclerosis [35].

Patients with ocular cystinosis (formerly termed “adult” or “benign” cystinosis; OMIM 219750) never exhibit renal failure or retinal hypopigmentation, but have cystine crystals in their bone marrow and corneas [6]. They do have measurable residual cystine-transporting capacity in their polymorphonuclear leukocytes’ lysosomes [36]. The only clinical manifestation of ocular cystinosis is photophobia, and patients are generally diagnosed incidentally on eye examination that includes the use of a slit lamp. There exist approximately 20 ocular cystinosis patients, who have either one mild and one severe or two mild *CTNS* mutations [37]. The distinction among the three types of cystinosis is in part artificial, since there exists a continuum of disease severity rather than discrete categories.

Early Clinical Manifestations

As for patients with several other lysosomal storage disorders, individuals with cystinosis appear entirely normal at birth. Nevertheless, the disease eventually affects nearly every tissue of the body, with variable times of onset. The signs and symptoms can be described as early and late findings, demarcated roughly by adolescence. The earliest manifestations of cystinosis involve complications of renal Fanconi syndrome and growth retardation [16].

Renal Tubular Fanconi Syndrome with Rickets

Cystinosis is the most common identifiable cause of renal Fanconi syndrome in early childhood (see ▶ Chap. 36, “Pediatric Fanconi Syndrome”). Although the proximal tubulopathy does not respond well to cysteamine, cystinosis is a treatable disease and, therefore, should be considered first when renal tubular solute wasting is recognized. In cystinosis, Fanconi syndrome is not evident at birth but generally appears at 6–12 months of age, with variable severity. In the past, and even now, in low-resource countries, infants have died of the dehydration and electrolyte imbalance associated with the Fanconi syndrome, without the benefit of a diagnosis. The Fanconi syndrome of cystinosis is nearly always complete and includes failure to reabsorb water, metabolic acidosis, electrolytes disturbances (hypokalemia and occasional hyponatremia), urinary losses of minerals (phosphaturia, hypocalcemia, and hypomagnesemia), amino acids, carnitine, glucose, and small-molecular-weight proteins (see chapter Fanconi syndrome). The clinical presentation of the Fanconi syndrome includes polyuria (often 2–3 L per day in small children and sometimes up to 5–6 L per day), dehydration (sometimes with consequent fevers), and polydipsia. Large volumes of fluid intake fill the stomach and reduce appetite. Phosphate and calcium wasting cause rickets, with low serum phosphate and calcium, and elevated alkaline phosphatase levels. In some cases, significant hypocalcemia triggers secondary hyperparathyroidism, exacerbating bone reabsorption. Children may fail to walk because of the bone pain of rickets, and they exhibit tender, swollen wrists and ankles due to metaphyseal widening. In severe cases, frontal bossing, a rachitic rosary, and genu valgum/varum develop. Osteoporosis and epiphyseal fraying are visible on radiographs. The combination of hyperphosphaturia and hypercalciuria may result in medullary nephrocalcinosis. Hypocalcemia can cause painful episodes of tetany or even seizures, especially 20–30 min after a dose of an alkalinizing medication that lowers circulating concentrations of ionized calcium.

Glomerular Damage

Cystinosis accounts for 1–5% of chronic renal failure in children [38, 39]. By the time a typical infant with cystinosis is diagnosed at approximately 1 year of age, significant renal damage has already occurred. It has been postulated that rapid progress to renal damage could be explained by inflammation caused by cystine crystals [40]. Early podocyte injury is likely to coexist along with proximal tubular dysfunction contributing to renal disease progression [41]. A reasonable estimate would place the creatinine clearance at the time of diagnosis at approximately 70% of normal, and this level of function slowly but inexorably decreases. Without treatment, most children with cystinosis have reached end-stage kidney disease (ESKD) by age 10 and require transplantation or dialysis. In a European study of 205 children with cystinosis, the mean age for ESKD was 9.2 years. Chronic kidney disease in cystinosis resembles that of other renal disorders, but growth retardation, osteodystrophy, and anemia are often more severe. Blood pressure may increase as kidney function declines, but cystinosis per se is not a risk factor for developing hypertension.

Growth Impairment

Newborns with cystinosis exhibit normal height, weight, and head circumference. While head circumference is maintained, height and weight percentiles generally fall by 6–12 months of age; failure to thrive is often the first indication of the diagnosis. By 1 year of age, the average infant with cystinosis has a height at the third percentile [42]. Without treatment, growth continues at 50–60% of the normal rate (Fig. 3). Weight usually follows height, but with greater variability. In patients not receiving growth hormone therapy, bone age usually lags behind chronological age by 1–3 years. Children with retarded bone age retain growth potential past the usual age of epiphyseal closure, but height is never gained past age 20, regardless of the bone age. The cause of impaired growth has not been definitively determined. Growth hormone levels are usually normal [43], but patients may have abnormal kinetics of growth hormone secretion after glucagon stimulation [44] and respond to supraphysiologic doses of recombinant growth hormone [45]. Hypophosphatemic rickets, acidosis, poor nutrition, and renal insufficiency probably all contribute to the poor growth of cystinosis. In addition, cystinosis patients develop a distinct form of chronic kidney disease–mineral and bone disorder (CKD-MBD), characterized by lower levels of fibroblast growth factor-23 (FGF-23) and

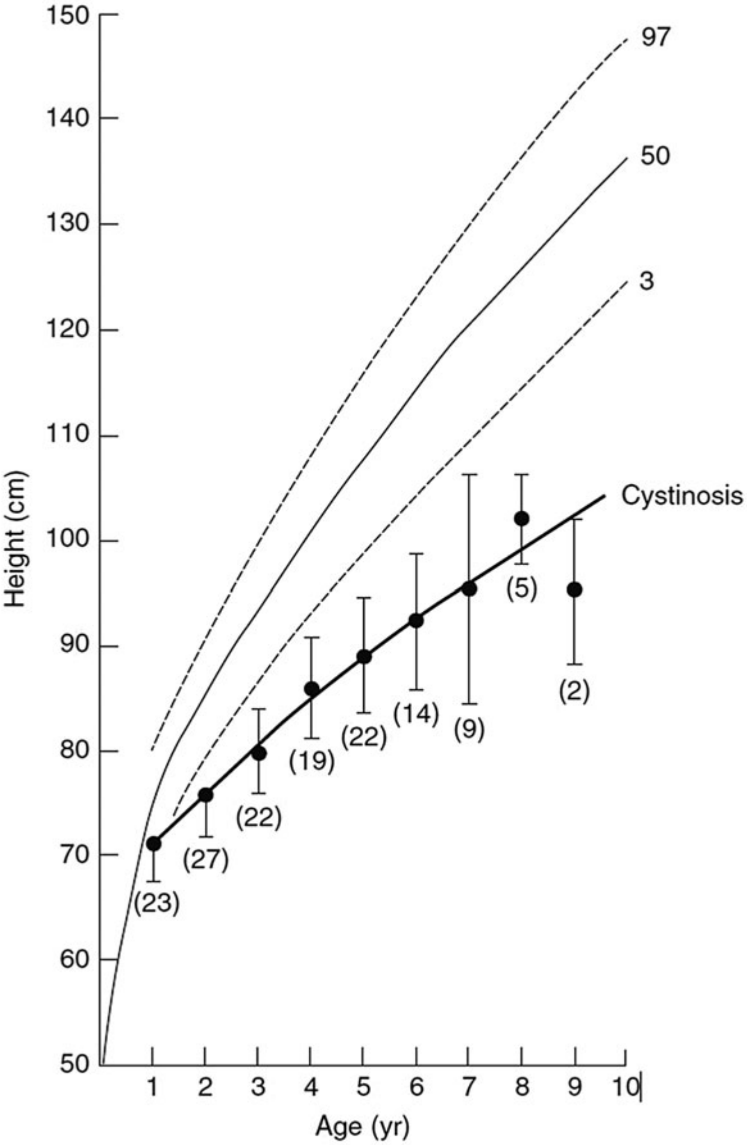


Fig. 3 *Natural history of growth in children with cystinosis*. Mean values for cystinosis children are superimposed upon a normal growth chart. On average, a child with cystinosis falls from normal height to the third percentile at 1 year of age and continues to grow slowly so that the height age is 4 years when the chronological age is 8 years. (Reprinted from the New England Journal of Medicine [42])

parathyroid hormone (PTH), even in advanced stages, that are related to persistently decreased renal phosphate reabsorption [46, 47, 48]. Patients with nephropathic cystinosis are, on average, shorter than children with similar degrees of kidney failure due to other causes.

Ocular Involvement

The primary ophthalmic manifestation of cystinosis is photophobia. This occurs due to corneal crystals that first appear in the anterior third of the cornea [49]. Corneal crystals are always present by 16 months of age on slit lamp examination and are diagnostic for cystinosis [50]. The number of crystals increase with age, reaching a maximum density by 8 years of age. An atlas of corneal crystal density at different ages has been published [50]. In vivo confocal microscopy (IVCM) and anterior-segment optical coherence tomography (AS-OCT) allow for more accurate quantification of corneal crystal deposition [49, 51]. Crystal density among patients with ocular cystinosis appears less than that in patients with classical disease. Children with cystinosis complain of sensitivity to light at variable ages, but usually not until 5–10 years of age. They squint to the point that, without treatment, they can eventually develop blepharospasm refractory to all modes of therapy. Often, patients wear dark glasses outside and turn down the lights inside. Occasionally, a child with cystinosis may experience a corneal ulceration as a crystal breaks through the corneal epithelium. This complication, which resolves spontaneously, occurs much more frequently in adolescence and adulthood, when haziness of the cornea often appears, but has become very rare in patients who have been treated with topical and systemic therapy since early in life.

Hypothyroidism

In the natural history of cystinosis, approximately half of patients exhibit hypothyroidism by age 10 [52] and 90% by age 30 [53], with low thyroxine and free T4 levels and elevated TSH levels. In cystinosis, the thyroid tissue appears fibrotic with occasional crystals present. In mice with cystinosis, hyperplasia and hypertrophy of thyroid follicles precede colloid exhaustion [54]. Treatment with cysteamine remarkably delays and possibly prevents the development of hypothyroidism.

Cognition and Psychological Aspects

Children with cystinosis learn normally and have low normal full-scale IQs [55–57]. However, isolated deficits in visual processing [58], tactile recognition [59], and short-term visual memory have been reported [59]. The neurocognitive profile is further characterized by significant discrepancies between perceptual reasoning and verbal comprehension, and impairment of working or auditory sensory memory [57]. Brain MRI shows cortical and subcortical brain atrophy, especially in parieto-occipital regions, resulting in lower total IQ scores observed in patients with atrophic brains [57, 60]. Patients with cystinosis may exhibit behavioral and social problems, which are related in part to their chronic disease, burden of therapy, and uncertainty of prognosis [60, 61].

Other Clinical Findings

Children with cystinosis are notoriously poor eaters, with craving for salty foods such as pickles, ketchup, and potato chips. In addition, many children exhibit a pica for hot foods such as jalapeno peppers, and tabasco sauce, possibly related to the functional loss of the capsaicin- and heat-sensitive ion channel TRPV1 in patients harboring the 57-kb deletion [62]. Young children and infants have an increased tendency toward vomiting, which is more severe in the morning prior to eating. Some of this may be related to medications. Excessive water intake with increased thirst causes abdominal distention and a sense of fullness, which further contributes to nausea, vomiting, and poor appetite. The nausea and vomiting often decrease with age and generally cease by 7–8 years of age. Rarely, a patient may suffer from decreased gastrointestinal motility and have projectile vomiting upon eating or drinking. One-third to one-half of children 10–18 years of age have mild hepatomegaly on physical examination [52], with no identifiable cause. Patients from lightly pigmented backgrounds appear less pigmented than other family members. This probably reflects dysfunction of melanosomes, which are lysosome-related organelles [63]. Patients of African or Asian descent have pigmentation, but often slightly less compared to their siblings. Most patients manifest decreased sweat production, causing flushing, heat avoidance, and occasional hyperthermia [64]. In addition, tear and saliva production is often reduced in cystinosis. Enuresis in children with cystinosis may reflect the large volume of urine produced daily. Idiopathic intracranial hypertension, or pseudotumor cerebri due to non-absorptive hydrocephalus, has been reported in several patients and has been attributed, in part, to cystinosis itself [65, 66]. Rarely, optic nerve compression due to idiopathic intracranial hypertension has caused blindness in cystinosis.

Diagnosis

Because a safe and efficacious treatment exists for cystinosis, physicians should maintain a high index of suspicion in patients demonstrating any characteristic findings. Unfortunately, the average

age of diagnosis for cystinosis remains just over 1 year, and even today several patients escape detection for years despite manifesting typical signs and symptoms.

Postnatal Diagnosis

A family history of cystinosis will naturally point to this disease in a child with suggestive findings. However, even without a previously affected sibling, evidence of renal tubular Fanconi syndrome should prompt investigation for cystinosis. Other signs include poor growth, failure to walk at an appropriate age, or evidence of rickets. The presence of typical corneal crystals on slit lamp examination by an experienced ophthalmologist will establish the diagnosis. Such crystals are usually not apparent within the first several months of life, but crystals are always present by 16 months of age [50]. Elevated intracellular-free cystine concentrations also are diagnostic. Patients have high concentrations of cystine in a variety of cell types, but polymorphonuclear leukocytes are the preferred cells in which to perform measurements [6, 13, 16, 67, 68]. While affected lymphocytes have three- to fivefold elevations above normal cystine concentrations, neutrophils have 30–100 times the normal levels, i.e., 3–23 nmol half-cystine/mg protein (normal, <0.2) [69]. Units of half-cystine are employed by convention, because early assays did not distinguish cystine from cysteine. Patients with intermediate cystinosis generally have values at the lower end of the nephropathic range, and patients with ocular cystinosis have values of 1–5 nmol half-cystine/mg protein [70]. White blood cell heterozygous values are less than 1 nmol half-cystine/mg protein. Several methods for intracellular cystine analysis have been used in the past, but these has been progressively replaced by liquid chromatography–tandem mass spectrometry [71, 72]. Approximately 3–10 mL of acid-citrate blood is required to isolate polymorphonuclear leukocyte-rich fractions. Red blood cell and lymphocyte contamination increase the protein in the denominator, causing underestimation of the cystine concentration. Sample storage at room temperature for >12–24 h may also cause spuriously low values. Immuno-magnetic beads allow isolation of granulocytes up to 30 h after blood sampling [73]. Limited sensitivity of the assay is required for diagnosis, but higher sensitivity is necessary for monitoring treatment efficacy. In the past, tissue biopsies (kidney, bone marrow, rectal mucosa, or conjunctiva) were used for diagnosis, but are no longer indicated. *CTNS* gene mutation analysis confirms the diagnosis; multiplex PCR allows rapid identification of the common 57,257-bp deletion. Cystinosis is a monogenic disease. The mutation detection is very high. Very few intronic mutations requiring sequencing of mRNA products for diagnosis have been reported [74]. Newborn screening allows for early diagnosis and treatment, which should result in improved long-term prognosis [75]. Two methods have been proposed: (a) tandem mass spectrometry for the determination of derivative seven-carbon (C7) sugars in dried blood spots (DBS) that can only detect homozygous 57-kb deletions of the *CTNS* gene and (b) molecular testing using next-generation sequencing techniques that allow detection of the most common *CTNS* mutations [76, 77].

Prenatal Diagnosis

Cystinosis can be diagnosed prenatally. When the mutation is known, molecular diagnosis can be performed on chorionic villus samples or on circulating fetal nucleated cells. Chorionic villus cells can also be assayed directly for cystine content, if enough material is available [78, 79].

Heterozygote Detection

Carrier (heterozygote) detection by leukocyte cystine measurement may be problematic. While carrier levels can be as high as 1 nmol half-cystine/mg protein, they can also be within the normal range, especially when cystine is measured on mixed white blood cells and not on purified granulocytes [67]. Molecular diagnosis is the preferred method to diagnose carriers.

Differential Diagnosis

Polyuria, hypokalemia, and glucosuria associated with renal Fanconi syndrome can sometimes lead to the mistaken diagnosis of nephrogenic diabetes insipidus, Bartter syndrome, or diabetes mellitus. Once true Fanconi syndrome is diagnosed, cystinosis should be considered first, before considering other causes of proximal tubular dysfunction (see ▶ Chap. 36, "Pediatric Fanconi Syndrome"). The vitamin D–resistant hypophosphatemic rickets of cystinosis has been mistaken for vitamin D deficiency or for other genetic forms of rickets (see ▶ Chap. 40, "Renal Hypophosphatemia"). Rarely, patients have carried the diagnosis of FSGS or simply kidney insufficiency until crystals were discovered upon removal of the native kidneys at transplant. Conversely, cystinosis was mistakenly diagnosed in a patient with idiopathic nephrocalcinosis but without intracellular cystine storage. Cystinosis should not be confused with cystinuria, in which cystine, arginine, ornithine, and lysine fail to be properly reabsorbed by renal tubular cells (see ▶ Chap. 54, "Urinary Stone Disease and Nephrocalcinosis"). In cystinosis, generalized aminoaciduria occurs because of the Fanconi syndrome, but there is no particular elevation in urinary cystine, and no diathesis toward renal stone formation, except in rare cases with severe hypercalciuria and nephrocalcinosis.

Therapy

Treatment of nephropathic cystinosis can be divided into symptomatic modalities for particular organ complications and cystine-depleting therapy directed specifically at reducing the cystine content of all cells of the body.

Replacement of Renal Losses

Renal tubular Fanconi syndrome results in wasting of small molecules, which need to be replaced (also see ▶ Chap. 36, "Pediatric Fanconi Syndrome"). First, there must be free access to water and bathroom privileges. Dehydration can accelerate the progression of renal failure. At the same time, electrolytes must be supplied. Sodium should not be restricted, and affected children should be permitted to indulge their natural cravings for salty foods. Potassium generally needs to be supplemented as either chloride, gluconate, or citrate salts. For alkalinization, citrate or bicarbonate salts of potassium or sodium can be used [80]. Phosphate is provided as potassium or sodium salts. Electrolytes, alkali, and phosphate salts should be administered every 6 h to ensure stable plasma levels. Vitamin D, as calcitriol or alfacalcidol in therapeutic doses, fosters gastrointestinal absorption of phosphate and calcium, although patients are not naturally deficient in vitamin D. With adequate phosphate and calcium replacement, monitored by serum alkaline phosphatase levels, rickets generally resolves in 3–6 months, although osteoporosis may persist on radiography. Children with hypocalcemia require calcium to treat their rickets and to prevent tetany. If calcium supplementation is required, care must be taken to separate the administration of calcium and phosphate by at least 60 min to prevent precipitation of calcium phosphate in the gut [81]. Rarely, patients require magnesium and oral carnitine supplementation. Doses of replacement medications should be monitored regularly in growing children. After the adolescent growth spurt, decreased amounts of supplemental phosphate are generally required to minimize the development or progression of nephrocalcinosis [82]. Dehydrated children with cystinosis require large amounts of fluid and electrolytes for effective rehydration. As chronic kidney failure progresses, cystinosis patients usually do not become oliguric, hyperkalemic, or hyperphosphatemic, until reaching advanced disease or end-stage kidney failure.

Other Symptomatic Treatments

The poor eating habits, nausea, vomiting, and early satiety due to excessive fluid ingestion all contribute to poor nutrition, especially around the time of diagnosis. Nutritional consultations can prove critically important to ensure, for example, that ingested fluids contain calories whenever possible. Many (but not all) infants and toddlers require feeding tubes to achieve adequate nutrition and sufficient hydration, and there should be a very low threshold to inserting feeding tubes. Once mineral, electrolyte, and acid–base balance are established, children can begin to eat normally. Most children eat well by 6–10 years of age and do not require feeding tubes. An occasional child requires gastric tube access for medications, hydration, or calories, but maintenance of the masticatory process is very important. Some nephrologists prescribe angiotensin-converting enzyme inhibitors to reduce proteinuria, based upon the potential benefit of this therapy in maintaining renal function in many chronic kidney diseases. The efficacy of this practice with respect to glomerular function has not been proven in cystinosis. If prescribed, angiotensin-converting enzyme inhibitors should always be used prudently, especially in patients with severe polyuria, and not in association with indomethacin to avoid compromising renal perfusion. Some patients, especially those in Europe, receive indomethacin to reduce urine volume [80, 83]. Indomethacin administration is generally reserved for young patients, particularly if they have huge urine volumes and severe growth retardation. In the past, most patients with cystinosis required thyroid hormone replacement, but treatment with cystine-depleting therapy has reduced the number of patients who require L-thyroxine supplementation [53, 84]. Some children benefit from judicious use of anti-emetics, which are most often taken just before the breakfast meal. The impairment of sweating predisposes to heat prostration, so children should avoid prolonged periods in the sun. Exercise helps to maintain muscle strength in the face of nutritional challenges. Skeletal deformities should be addressed in a timely fashion, and patients with severe rickets or renal osteodystrophy may require corrective orthopedic surgery such as an osteotomy [81]. Physical therapy is critical for such patients to correct abnormal body alignment, maintain muscle tone, and prevent further skeletal abnormalities such as scoliosis later in life. Although patients possess normal amounts of growth hormone, they may still benefit from growth hormone supplementation if GFR is above 50 ml/min/1.73 m [45]. Caution should be used however, in prescribing growth hormone in children with hyperparathyroidism. Supplementation of renal losses, appropriate caloric intake, and cysteamine therapy (see below) can achieve normal growth rate within 6–12 months, although it will not usually allow for catch-up growth. Hence, if children remain below the third percentile for height a year after their medications have been adequately received, growth hormone should be considered [81].

Renal Replacement Therapy and Kidney Transplantation

No particular findings distinguish patients with cystinosis from other children and young adults requiring renal replacement therapy. Once a person with cystinosis reaches ESKD, renal replacement therapy with hemodialysis, peritoneal dialysis, or renal transplantation is required. Cysteamine clearance during dialysis does not require dose adaptation [85]. With specific regard to renal transplantation, carriers of *CTNS* mutations are suitable candidates as kidney donors. Cystinosis does not recur in the transplanted kidney, because the lysosomal cystine transport defect is intrinsic to the host cells. Nevertheless, a graft biopsy can sometimes contain cystine crystals due to infiltration of the donor kidney by host macrophages. Overall kidney graft survival is excellent and is, on average, superior to that for other conditions [86, 87]. Nonetheless, patients with cystinosis should receive standard antirejection therapy. Steroid-free immunosuppression is recommended in patients without additional risks of rejection. While current practice recommends retention of host kidneys, if some native kidney function remains, blood flow to the host kidneys may result in persistence of the Fanconi syndrome post-transplant. Polyuria may occasionally be problematic, and potassium supplementation may be needed after renal transplantation. Most of the renal osmotic load, however, is handled by the transplanted kidney and losses from native kidneys usually resolve in the first months following transplantation. After a renal allograft, patients virtually always improve in activity, appetite, and feeling of well-being. Patients grow significantly after transplantation, at rates that depend upon chronological age and bone age.

Oral Cysteamine Therapy

Many putative cystine-depleting regimens have proven unsuccessful in cystinosis patients, including restriction of sulfur-containing amino acids and the administration of ascorbic acid, dithiothreitol, and penicillamine [88]. One specific radioprotective agent, however, has proven efficacious. In 1976, the aminothiol cysteamine, or beta-mercaptoethylamine [89], was shown to deplete affected fibroblasts in vitro, and polymorphonuclear leukocytes in vivo, of cystine. Cysteamine passes through the plasma and lysosomal membranes into the lysosome, where the acidic environment puts a charge on the amine group and traps the molecule. Within the lysosome, cysteamine interacts with cystine in a disulfide interchange reaction to produce cysteine and cysteine–cysteamine mixed disulfide, both of which readily exit the lysosome without requiring a functional cystine transporter (Fig. 4). Cysteine can exit lysosomes through its own carrier, while the mixed disulfide exits through the cationic amino acid transporter PQLC2 [90]. In the cytosol, cysteine participates in normal cytoplasmic reactions and can be eliminated from cells.

When taken orally, cysteamine removes up to 95% of leukocyte cystine within 1 h of ingestion. Parenchymal cells are thought to respond in a similar fashion, although the extent and time course of depletion remains unknown for other tissues. A clinical trial of oral cysteamine therapy was initiated in 1978 [42]. By the end of the study in 1985, the cysteamine-treated group had significantly better kidney function than the untreated group (39 vs. 30 mL/min/ 1.73 m^2), despite being

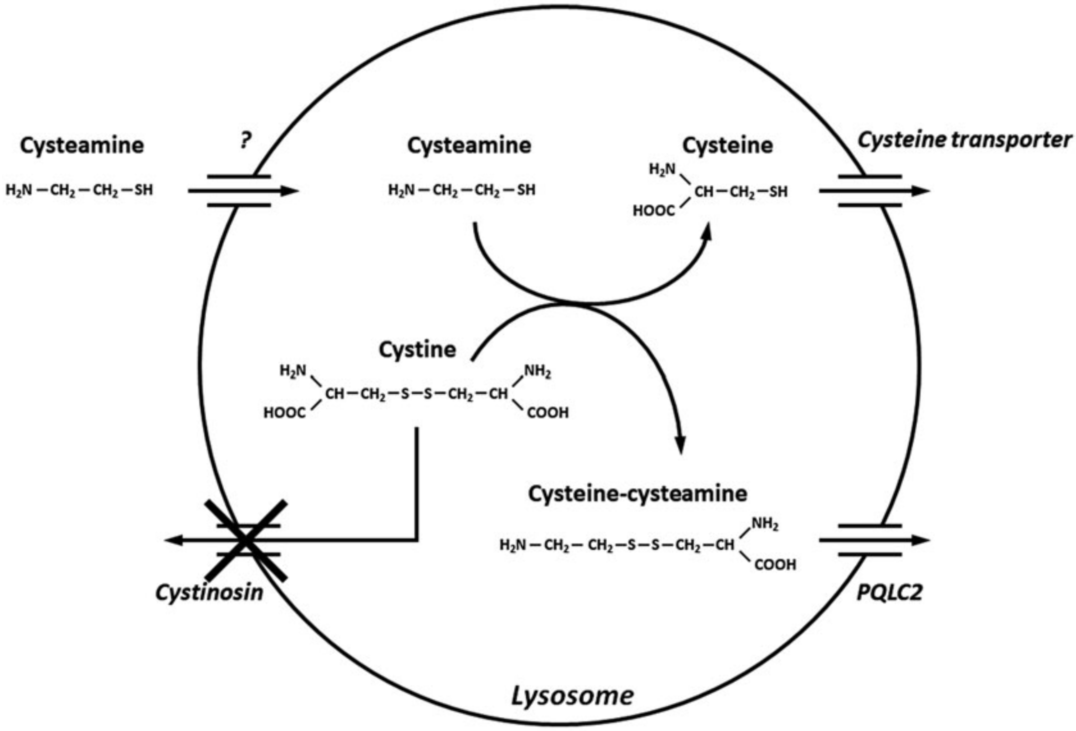


Fig. 4 *Mechanism of action of cysteamine*. The free thiol enters the lysosome and reacts with cystine (Cys-Cys), which cannot exit the cystinotic lysosome. The two products, cysteine and cysteine–cysteamine mixed disulfide, can readily leave the cystinotic lysosome; the mixed disulfide leaves through the PQLC2 transporter. This process lowers lysosomal cystine levels by over 90%

1.4 years older. Growth velocity was also better in the cysteamine-treated group. Based on this report, oral cysteamine became the treatment of choice for patients with nephropathic cystinosis prior to transplantation. Subsequent studies, including a seminal intent-to-treat analysis in 1993 [91], verified the safety and efficacy of chronic oral cysteamine therapy and demonstrated that patients treated early and effectively had a mean creatinine clearance of 57 mL/min/1.73 m^2 at a mean age of 8.3 years. At the same age, 60% of children without cysteamine treatment had progressed to ESKD and children with suboptimal treatment had outcomes between these extremes (Fig. 5). Cysteamine not only slowed the deterioration of glomerular function, but also allowed for normal increase of kidney function in the first 3 years of life [91]. Consequently, early initiation of cysteamine was deemed critical; every month of treatment in the first 2 years of life translates into 14 months of preservation of kidney function later in life. The optimally treated patients also grew at a normal rate, parallel to normal height curves, while remaining below the third percentile on average [6].

Cysteamine bitartrate was approved for treatment of cystinosis as Cystagon® in 1994 and 1997 in the USA and Europe, respectively. Cystagon® is given at a dose of 60–90 mg/kg/day or 1.3–1.95 g/m^2/day, in divided doses every 6 h

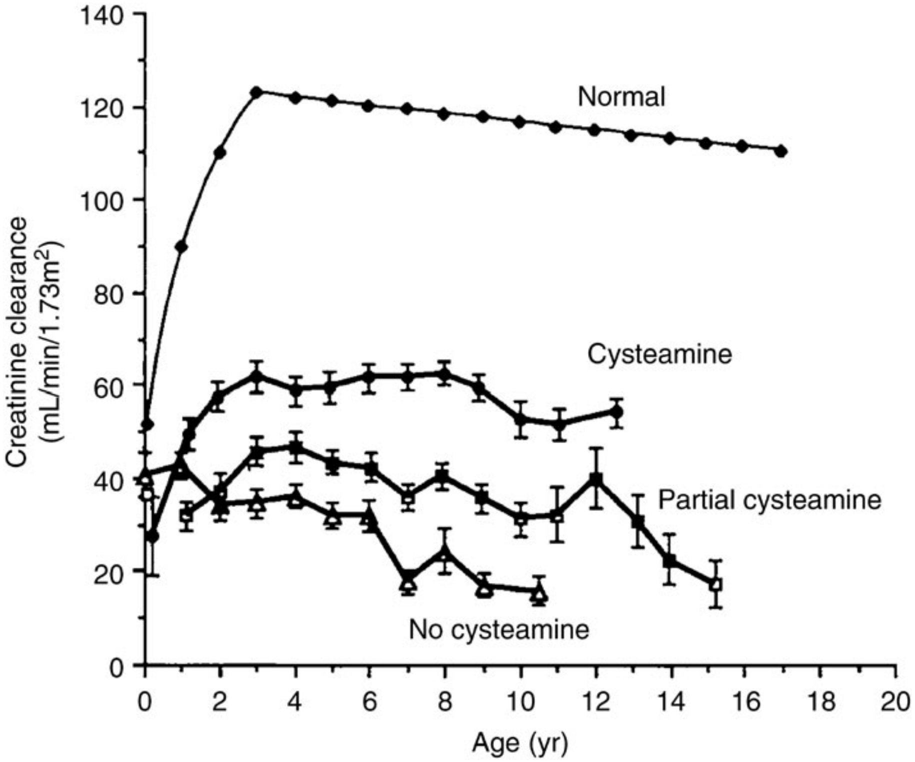


Fig. 5 *Cysteamine preserves renal glomerular function in cystinosis and allows for growth of the kidneys in the first 3 years of life*. Creatinine clearance, based upon 24-h urine collections, was used as a measure of glomerular filtration rate. The normal curve indicates rapid growth of renal function early in life as the kidneys enlarge. The other curves were constructed using values from children with cystinosis. The "No Cysteamine" curve reflects the natural history of cystinosis, i.e., a monotonic decline in creatinine clearance leading to end-stage renal disease at 10 years of age. The "Cysteamine" curve represents mean values for 17 children treated early and well with oral cysteamine. Renal growth and acquisition of increased renal function were preserved. The "Partial Cysteamine" curve was for patients treated less well, and the results fall between those in the other two groups. (Reprinted from the New England Journal of Medicine [91])

Table 1 Cysteamine therapy

	Drug	Dose	Frequency	Notes
SYSTEMIC TREATMENT ***cysteamine bitartrate***	Immediate-release (Cystagon®)	< 12 years of age: 1.30 – 1.95 g/m^2/day > 12 years of age: 2 g/day	q6h	*Start at 1/6th of the target dose and increase gradually over 6-8 weeks. Do not exceed max dose of 1.95 g/m^2/day. Monitor regularly leucocyte cystine levels. Add proton pomp inhibitor if gastric symptoms.*
	Extended-release (Procysbi®)	Start with 80% of the suggested dose for the immediate-release formulation	q12h	
OCULAR TREATMENT ***Cysteamine hydrochloride***	0.44% aqueous solution (Cystaran®)		q2h	*Ophthalmological evaluation every 6-12 months. Add artificial tears if dry cornea.*
	0.37% ophthalmic gel (Cystadrops®)		q6h	
	0.55% pharmacy-made solutions		q1h	

(Table 1). Blood levels peak at 1 h and fall off over the next few hours. Since children often have to take several pills, it is recommended to take the full dose rapidly, and the dose should be repeated if vomiting occurs within 15 min of ingestion. When initiating cysteamine therapy, dosing should begin at approximately one-sixth to one-fourth of the target dose, with incremental increases every 3–7 days. Doses are titrated to maintain leukocyte cystine levels less than 1.0–1.5 nmol half-cystine/mg protein, according to the reference value of the laboratory and the type of cells that are analyzed (i.e., whole peripheral leucocytes or granulocytes). The recommended adult dose of Cystagon® is 500 mg every 6 h, although some patients receive as much as 750 mg every 6 h. Despite its very beneficial effects on kidney function, early initiation of cysteamine therapy can delay but cannot avoid the need for kidney transplantation entirely [91]. Recent long-term registry data indicated that the overall survival of native kidney function during the last decennia was 5–10 years prolonged compared with historic cohort of patients who were not treated with cysteamine (Emma et al., unpublished data; ref. (87)). Furthermore, although cysteamine therapy can occasionally be associated with improved renal tubular function [92], the Fanconi syndrome of cystinosis is generally irreversible and refractory to cystine-depleting therapy. Some affected siblings of cystinosis patients have benefited from early diagnosis and started cysteamine before 2 months of age. In these patients, the Fanconi syndrome was delayed and attenuated but not entirely prevented. In one child identified through a neonatal screening, renal Fanconi syndrome had not developed at the age of 16 months [27].

Side effects of cysteamine preclude its use or decrease compliance to treatment in a substantial proportion of patients, especially during adolescence [42, 93]. Because cysteamine is a free thiol, it smells like rotten eggs, tastes bad, and can cause nausea and vomiting. A small fraction of cysteamine is metabolized into methanethiol and dimethylsulphide [94]. Although these metabolites are produced from less than 5% of ingested cysteamine, they are volatile, are eliminated in breath and sweat, and cause most of the bad smell that patients find offensive [94]. Breath fresheners, riboflavin, chlorophyll, and other over-the-counter remedies can help combat the foul smell. Changing clothes regularly during the hot season is also helpful. Anti-emetics can be useful for the nausea and vomiting.

Cysteamine is a potent gastric acid secretagogue; it has been used experimentally to induce duodenal ulceration in animal model. Oral cysteamine produces a fourfold increase in gastric acid production and a 50% increase in serum gastrin levels, which cause frequent gastrointestinal complaints, including nausea, dyspepsia, and epigastric pain [95]. Treatment with proton pump inhibitors is effective in reducing gastric acid hypersecretion and the risk of ulceration [95, 96]. Very rarely, patients may develop bruiselike lesions on the elbows corresponding to reactive angioendotheliomatosis on skin biopsy, striae, bone pain, and/or myalgia [97]. These lesions have been observed primarily among children on high doses of cysteamine, possibly associated with copper deficiency and interfering with collagen cross-linking [97, 98]. Other infrequent side effects include drug-induced lupus [99, 100], neutropenia, seizures, lethargy, somnolence, and drug rash [96].

Direct administration of cysteamine into the small intestine results in higher plasma cysteamine concentrations and prolonged drug exposure, compared to administration in the stomach or in the colon [101]. This allows maintenance of leucocyte cystine concentrations below trough levels for up to 12 h [102]. In 2013, an enteric-coated delayed-release (DR) formulation of cysteamine bitartrate was approved for clinical use in the USA and Europe as Procysby®. This formulation is administered every 12 h and is composed of enteric-coated capsules containing microspheronized beads that can also be pushed into feeding tubes. A phase III trial including 43 patients demonstrated noninferiority of the DR formulation in achieving leucocyte cystine depletion, compared with the standard IR formulation [103]. In addition, the trial showed that the DR formulation achieves similarly reduced leucocyte cystine levels with less cysteamine [103]. A two-year extension trial showed that mean leucocyte cystine levels remained within the therapeutic target range with stably reduced cysteamine doses [104]. Improvement in the social, school, and total function aspects of quality of life that were observed during the phase III trial persisted; kidney function and linear growth also remained stable during the two-year period [104]. Several patients treated with DR cysteamine have reported less halitosis [105]. Conversely, some patients have switched back to the IR preparation because they experienced more gastrointestinal symptoms with the DR formulation [105].

Compliance with cysteamine can be very challenging and is negatively influenced by side effects and by the frequency of administration [93, 106]. As for other chronic conditions, medication adherence often drops during adolescence; in the case of cystinosis, this generally leads to rapid deterioration of kidney function. Monitoring leukocyte cystine levels helps identify patients with poor adherence, although results reflect only short-term adherence to therapy. In this respect, serum levels of chitotriosidase, which is released as a result of macrophage activation by cystine crystals, may be more informative [107]. The clinical utility of this marker needs to be confirmed in long-term studies. Regardless of the monitoring strategy, constant patient motivation is essential. It is estimated that each year of good compliance increases kidney survival by nearly another year [108].

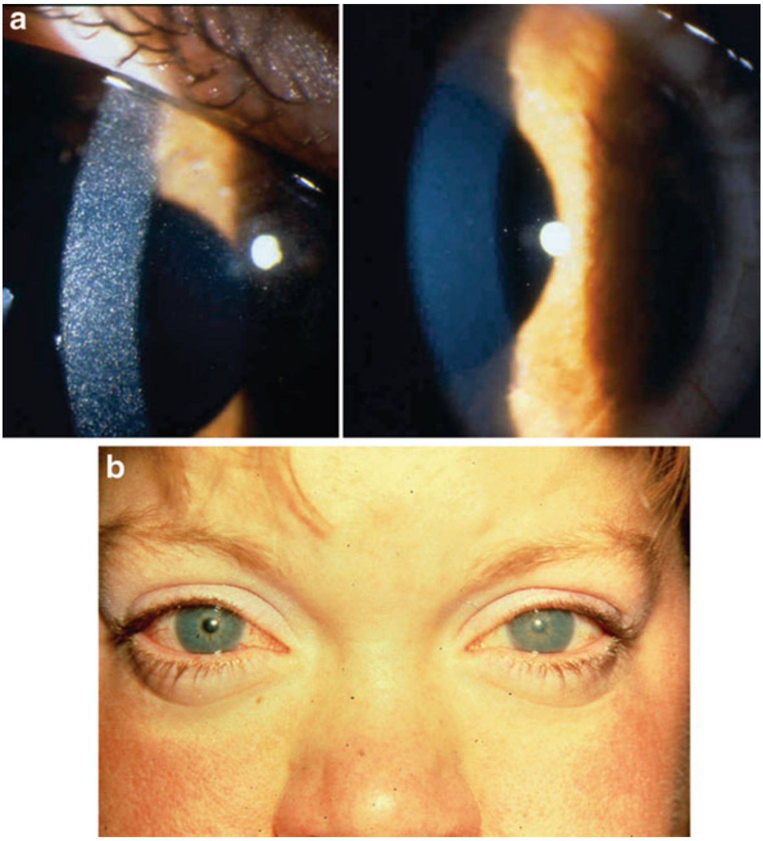
Cysteamine should not be prescribed to pregnant females because of its potential teratogenicity. Treatment should be discontinued immediately after conception and should not be resumed until after delivery. Since all reported pregnant patients were transplanted or treated with dialysis, stopping cysteamine during pregnancy has never been shown to compromise native kidney function [109]. However, cysteamine withdrawal represents a dilemma in well-treated women who reach childbearing age with preserved kidney function. For similar reasons, cysteamine is not used for prenatal treatment of affected fetuses.

Chronic oral cysteamine treatment has been shown to deplete the muscle and liver of cystine [110]. The inference that other tissues became similarly depleted is bolstered by evidence that treated patients escape the need for thyroid hormone replacement [53]. Recent evidence indicates that long-term oral cysteamine can prevent or delay most, if not all, of the late complications of cystinosis [34, 84]. Patients diagnosed with ocular cystinosis may, in fact, have a mild form of nephropathic cystinosis and may eventually develop glomerular dysfunction. Hence, such individuals should be monitored for any decline in kidney function [35].

Cysteamine Eyedrops

Oral cysteamine fails to reach the avascular cornea, so cystine crystals continue to accumulate

Fig. 6 *Corneal cystine crystals and the effects of cysteamine eyedrops*. (**a**) Left: slit lamp examination of a 21-year-old cystinosis patient showing the cornea packed with crystals. Right: Same cornea 3 years later, nearly devoid of crystals subsequent to application of topical cysteamine therapy. (**b**) An adult post-transplant female patient with cystinosis treated her right eye with cysteamine eyedrops, and the cornea became clarified. Her untreated left eye remained hazy, filled with cystine crystals



there despite cysteamine treatment. Delivery of cysteamine directly to the cornea, through cysteamine eyedrops, has proven effective. In general, ocular symptoms improve within approximately 3 weeks and the photophobia is at least partially relieved [111]. Regular application of the eyedrops can dissolve crystals in 6–12 months (Fig. 6). The beneficial effects of cysteamine eyedrops can occur at any age, and older individuals can observe clarification of the haziness in their corneas [50]. The disulfide cystamine is not as effective as the free thiol cysteamine in dissolving corneal cystine crystals [112]. Storage conditions may adversely affect the stability and efficacy of cysteamine. In many centers, hospital pharmacy–made solutions are used. These often cause discomfort related to the low pH of the solution, and are recommended to be used every hour during daytime. Most patients have difficulty in maintaining rigorous compliance. Recently, two commercial preparations have been introduced in the USA and in Europe. CYSTARAN® is a 0.44% cysteamine eyedrop solution with increased stability and tolerability [113], and CYSTADROPS® is a 0.55% viscous solution that can be applied four times per day and has shown long-term benefits on corneal crystal deposition [114, 115] (Table 1). Dry cornea is a frequent complication of ocular cystinosis; when present, artificial tears should be used in conjunction with cysteamine eyedrops. Some partial relief of corneal discomfort can be achieved by over-the-counter eyedrops, probably by virtue of their lubricating ability.

Other Therapeutic Considerations

Families must recognize that oral cysteamine therapy is lifesaving, and counseling in this regard should be part of the therapeutic plan. Vaccinations need to be handled diligently, particularly in post-transplant patients on immunosuppressive therapy.

Many children require physical, speech, and occupational therapy. By the time children with cystinosis enter school, they have interacted closely with health professionals for years and may appear sophisticated. However, they are still children who need limits as well as understanding. Conversely, some adults with cystinosis are small in stature, but should be treated as adults. Psychological help should be obtained in difficult situations.

Hematopoietic Stem Cell Transplantation

Promising results have been observed after transplantation of cystinotic knockout mice with isogenic wild-type bone marrow [116]. Despite the fact that cystinosin is a ubiquitous protein, transplanted animals showed a disproportionally high reduction in cystine content, considering the magnitude of cell engraftment, and preservation of kidney tissue damage [116]. This observation led to the discovery that hematopoietic stem cell–derived macrophages can produce tunneling nanotubes that allow transfer of cystinosin-bearing lysosomes into affected cells [117]. In addition to improving the renal phenotype of knockout animals, hematopoietic stem cells also preserved thyroid function and prevented corneal crystal formation in the same experimental model [118, 119]. In humans, the first allogenic hematopoietic stem cell transplantation was performed in a 16-year-old boy [120]. Unfortunately, the patient expired from infectious and hematological complications, but successful transfer of wild-type cystinosin and *CTNS* mRNA to the recipient cells, as well as decreased cystine crystal deposition in tissues could be demonstrated [120]. A phase I/II clinical trial (NCT03897361) to assess the safety and efficacy of gene-corrected autologous hematopoietic stem cells is underway.

Cystinosis in Adults

Table 2 Frequency of complications in 100 adults with cystinosis[a]

Finding frequency	(%)
Hypothyroidism	75
Male hypogonadism	74
Pulmonary dysfunction	69
Swallowing abnormality	60
Myopathy	50
Retinopathy	32
Vascular calcifications	31
Diabetes mellitus	24
Cerebral calcifications	22

[a] 92% of patients had received a renal allograft; many had not benefited from early treatment with cysteamine

The success of renal transplantation has created a population of adult patients with nephropathic cystinosis, and this situation has revealed the consequences of prolonged cystine accumulation [34, 121]. In untreated patients, cystinosis eventually damages or destroys nearly every tissue and organ of the body, with variable frequencies (Table 2). This multisystemic involvement presents a significant challenge to the effective transitioning of patients from pediatrics to internal medicine. The following is a description of the natural history of late cystinosis, without cystine-depleting therapy.

Growth and Appearance

Untreated or poorly treated adult cystinosis patients (males and females) average 144 cm in height (13 cm below the mean third centile for males and females) and 45 kg in weight (5 kg below the third centile for males and females) [34].

Myopathy

A large proportion of untreated adults with nephropathic cystinosis exhibit a distal vacuolar myopathy, beginning in the hands with loss of the interosseous muscles and the thenar and hypothenar eminences and progressing to the arms, legs, shoulders, neck, and chest [23, 122]. This complication does not occur before adolescence and is attributable to cystine accumulation within muscle tissue, not to carnitine deficiency, which

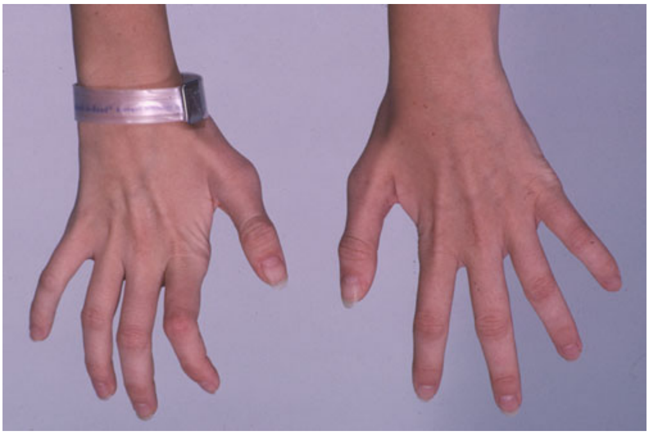


Fig. 7 *Myopathic right hand of an adult with cystinosis, untreated with oral cysteamine*. Note muscle wasting and clawhand posture of flexion

resolves with renal transplantation. Affected patients hold their fingers in partial flexion, giving the appearance of a claw hand (Fig. 7); they have difficulty opening jars. Without cysteamine treatment, the muscle weakness and atrophy progress inexorably to include the head and neck, causing poor tongue and lip strength and hypophonic speech. Nerve conduction velocities are normal, and electromyography shows a myopathic pattern [123]. Eventually, swallowing difficulty supervenes in most untreated myopathic patients. Symptoms include slow eating, choking, pain on swallowing, and heartburn [124]. The impairment results from deterioration of swallowing muscles rather than from nerve involvement, and it poses a significant risk of aspiration. The swallowing problem involves pooling in the vallecula and pyriform sinuses and, rarely, a double bolus. Other issues of oromotor dysfunction include a hyperactive gag reflex, hoarse voice, and inability to elevate the palate and move the jaw normally. Another concomitant of severe myopathy in cystinosis is restrictive lung disease with impaired respiratory function, due to weakness of the thoracic musculature rather than parenchymal lung damage [125]. Pulmonary function tests are often significantly reduced, with volumes approximating 50% of predicted for size and age. Expiratory muscle strength training programs may help to slow the progression of lung disease and decrease the risk of chronic aspiration [126]. Rickets during childhood might also confer a conical shape to the chest cavity, potentially reducing maximum achievable volumes.

Pancreatic Involvement

A significant number of post-transplant cystinosis patients have insulin-dependent diabetes mellitus [127, 128]. The frequency and severity of diabetes in cystinosis cannot be attributed entirely to the effects of steroids. Despite the fact that tacrolimus might contribute to the development of diabetes mellitus, most centers do not change their immunosuppressive regimen for cystinosis patients.

Rarely, pancreatic exocrine insufficiency has occurred in cystinosis, as evidenced by steatorrhea that is ameliorated by institution of pancreatic enzyme supplementation [129].

Hypogonadism

In the natural history of cystinosis, puberty is delayed by approximately 1–3 years, and some males never completely develop secondary sexual characteristics, including muscular development, facial hair, and a deep voice. Untreated adult male patients have low levels of testosterone and inhibin B and elevated levels of luteinizing hormone and follicle-stimulating hormone, reflecting testicular damage [130, 131]. If patients have been treated with cysteamine from early age, however, they may achieve normal sexual development and have normal sex hormone levels [131]. In most cases, sperm counts of subjects with infantile cystinosis show azoospermia, but some young patients may still have viable sperm cells in their ejaculate (unpublished data E.L.). Patients with intermediate or ocular cystinosis have oligospermia or normospermia, and can induce pregnancies (unpublished data E.L.). To date, one male patient with infantile cystinosis has fathered twins after testicular sperm aspiration combined with intracytoplasmic sperm injection [132]. Female patients have no apparent impairment of ovulatory function and have normal sex hormone levels. Several women with cystinosis have given birth to normal children [109]. Similar to other women with a kidney transplant or with chronic kidney failure, their pregnancy should be considered high risk and monitored carefully in specialized centers. As mentioned above, oral cysteamine therapy should be discontinued until after delivery.

Central Nervous System Involvement

Brain cystine levels were originally considered normal in cystinosis, and the brain was thought to be spared. This notion was discarded, however, as patients survived to adulthood by virtue of renal replacement therapy. In fact, many untreated adults with cystinosis have cerebral atrophy and periventricular and basal ganglia calcifications on CT scans [133]. These findings have not correlated directly or immediately with functional deficits. Reported neurological symptoms include seizures, memory and cognitive defects, stroke episodes, inability to walk, and loss of speech [134–137].

Ocular Findings

Untreated corneas of cystinosis patients continue to accumulate crystals and can develop a vision-impairing band keratopathy. Chronic photophobia can result in irreversible blepharospasm and posterior synechiae (scar tissue binding the iris to the posterior lens), impairing accommodation and causing angle closure glaucoma [21, 138]. Loss of retinal pigment epithelium can damage the rods and cones, initially impairing night and color vision and eventually reducing visual acuity. The retinopathy can be confirmed by electroretinography [139]. An estimated 3–5% of adults who have not received oral cysteamine therapy have retinal blindness.

Other Complications

Vascular calcifications occur with increased frequency among all individuals who have received dialysis, but the prevalence among cystinosis patients appears greater than expected [140]. Two young men with cystinosis died of severe liver dysfunction with portal hypertension; the pathologic diagnosis was nodular regenerating hyperplasia [25].

Death

In a study of 100 adults aged 18–45 years seen at the NIH Clinical Center between 1985 and 2006, 33 had died at an average age of 28.5 years [34]. In a second French study of 86 patients at least 15 years of age diagnosed between 1961 and 1995, 24 patients died at a mean age of 26.8 years. In both studies, lack of treatment with cysteamine was the major risk factor for early death.

Cross-References

▶ Pediatric Fanconi Syndrome

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Renal Tubular Acidosis

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Abstract

The kidneys are tasked to maintain homeostasis, and acid-base balance is a key part of this. They accomplish this task with nanomolar precision, as blood pH is maintained in a very tight range of 7.37–7.43, equivalent to a proton concentration around 40 nmol/l. As our diet typically presents an acid load, the equivalent of this load must be excreted in the urine to maintain balance. In addition, the formation of hydroxyapatite, an alkali needed for bone mineralization, generates an additional acid load that must be excreted by the kidneys. Together, these processes generate an acid load of typically 1–3 mmol/kg/day. Yet, before acid excretion can be accomplished, the kidney tubules must reclaim virtually all of the filtered bicarbonate, approximately 4000 mmol/day in an adult, as in the acid-base balance each molecule of bicarbonate lost is equivalent to one proton gained.

Reclaiming of filtered bicarbonate occurs predominantly in the proximal tubule, whereas protons are secreted in the distal tubule. Each of these processes involves a number of specialized enzymes and transport molecules, dysfunction of which can lead to renal tubular acidosis (RTA). Defects in bicarbonate reabsorption consequently are termed proximal RTA, whereas defects in proton secretion cause distal RTA. And while proximal RTA is difficult to treat, as, like in any tubular wasting disorder, supplementation primarily increases tubular losses, distal RTA can be treated relatively easy by providing alkali supplementation in an amount matching the acid load. In this chapter, we discuss the molecular basis of renal acid-base homeostasis, the different forms and etiologies of renal tubular acidosis, and their diagnosis and management.

Keywords

Renal tubular acidosis · Proximal renal tubular acidosis · Distal renal tubular acidosis · Bicarbonate · Urine pH

Introduction

Renal tubular acidosis (RTA) is defined as a group of tubular transport disorders affecting the reabsorption of bicarbonate (HCO_3^-), secretion of hydrogen ion (H^+), or both in the absence of a substantial impairment in the glomerular filtration rate [1]. The resultant biochemical abnormalities are characterized by a hyperchloremic/normal anion gap metabolic acidosis.

Clinical consequences of the acidosis include bone disease, such as rickets or osteomalacia due to buffering of acid by hydroxyapatite in the bone [2]. The excess calcium dissolved from the bone is excreted in the urine which can result in nephrocalcinosis and/or urolithiasis [3]. In children, growth failure is a common presentation [4].

RTA can be inherited (primary) or acquired (secondary). In this chapter, we review the various forms of RTA, the history, molecular basis, and the treatment.

Historical Development of the Classification of RTA

The study and understanding of the renal tubular acidosis began in the early twentieth century and has continued to evolve in the twenty-first century with the expansion of molecular biology.

In a British Paediatric Association meeting of 1935, Lightwood first described a group of six young children (from a large autopsy series) with what he described as “calcium infarction” of the kidneys [5]. This was later recognized as the first report of infants with nephrocalcinosis from renal tubular acidosis. Only a year later, Butler and colleagues described four infants with similar features and hyperchloremic acidosis, although the connection between acidosis and nephrocalcinosis was at that time unclear [6]. A decade later, Albright brilliantly provided what is considered the first pathophysiological description of RTA [7]. Key insights provided by Albright in his extensive essay on "osteomalacia and late rickets" included: a) an understanding of the disease as "renal acidosis resulting from tubular insufficiency without glomerular insufficiency," b) the understanding that acidosis causes hypercalciuria, c) the consequent link to nephrocalcinosis/urolithiasis, d) a correlation between this "renal tubular insufficiency" and the previously described familial periodic paralysis, and e) the treatment recommendation of these patients with a solution of sodium citrate previously designed by Alfred Shohl.

The term "renal tubular acidosis" was coined in 1951 [8].

During the following years, further research on the physiology of renal tubular acidification led to the development of different techniques to measure the capacity of the distal tubules for acid secretion. This identified "distal tubular insufficiency" as the main mechanism for the disease. In particular, the work of Wrong and Davies not only outlined what later on became the gold standard diagnostic protocol (ammonium chloride test), but also provided an extended phenotype with the description of the so-called incomplete renal tubular acidosis, defined as a failure to acidify urine in response to an oral acid load but with an absence of overt systemic acidosis in routine conditions [9].

Stapleton and colleagues in 1949 described patients with significant bicarbonaturia in a context of low serum bicarbonate [10]. This suggested that in some patients the disease mechanism was not impaired distal acidification but rather proximal reabsorption. Rodriguez-Soriano and Edelman subsequently proposed a new classification, based on the threshold for bicarbonate excretion and thereby distinguishing between proximal and distal RTA [1]. Later on, Morris and colleagues described another subtype, denominated type III, and characterized by a combination of proximal and distal RTA [11]. This was based on observations in two infants and most likely reflected the transient proximal tubular dysfunction commonly seen in patients with distal RTA at presentation, which resolves with treatment [4]. However, in the 1980s, a rare autosomal recessive syndrome presenting with osteopetrosis, combined proximal and distal renal tubular acidosis, cerebral calcification, and mental impairment was described and linked to pathogenic variants in *CA2*, encoding carbonic anhydrase type II, expressed both in proximal and distal segments and involved in bicarbonate reabsorption, as well as proton secretion [12].

The discovery of aldosterone did not only have a significant impact on our understanding of sodium and potassium physiology but also subsequently led to the recognition of a fourth type of renal tubular acidosis. This form was seen in patients with aldosterone insufficiency or resistance and is characterized by hyperkalemia rather than hypokalemia [13]. This form of RTA was then referred to as type IV RTA. It is important to recognize, however, that the primary defect here is a failure to reabsorb sodium in the collecting duct with the resultant "voltage defect" impairing acid secretion [14]. Thus, type IV RTA is primarily a salt-wasting disorder with a secondary defect in distal acidification.

Over the past century, advances in renal physiology, clinical investigations, and molecular genetics have greatly improved our understanding of the various forms of renal tubular acidosis. Currently, the diagnosis and classification of the various types of renal tubular acidosis continue to rely on biochemical measurements of blood and urine but are increasingly complemented by genetic testing.

Currently, more than 50 disease genes for kidney tubulopathies are recognized, and the list keeps on expanding. Because of the specific phenotype associated with mutations in most genes, an accurate clinical diagnosis can usually be established. However, even in expert centers, genetic testing can sometimes further specify or even correct the clinical diagnosis, so that genetic confirmation is usually recommended because of potentially important implications not only for genetic counseling, but also for treatment [15, 16].

Physiology of Acid Secretion

Extracellular pH is normally regulated within an extremely tight range (7.37–7.43), reflecting nanomolar changes in proton concentration. As protons can bind to amino acids like histidine, a change in pH will lead to a change in the charge of a given protein, which in turn can affect protein folding and function [17]. This highlights the critical importance of acid-base homeostasis for cellular function in general, and many of the non-specific clinical symptoms encountered in untreated patients with distal renal tubular acidosis such as fatigue and lack of appetite may reflect this.

A common western diet is usually generating a daily acid load of 1 mmol/kg of body weight [18], and growing children typically produce an extra 1–2 mmol/kg of body weight as a result of bone mineralization (generation of hydroxyapatite), which in total makes an approximate acid gain of 2–3 mmol/kg/day that the kidneys need to excrete [19, 20]. If the kidneys are not capable of eliminating this acid load, the body will need to use its existing buffers in order to neutralize the excess (bicarbonate, hemoglobin, phosphate, and other plasma proteins). The amount of acid excreted by the kidneys is referred to as net acid excretion (NAE) and is expressed quantitatively as:

$$NAE = \left(U_{NH_4} + U_{TA} - U_{HCO_3}\right) \times V$$

where V is the urinary flow rate, U_{NH4} is the urinary ammonium concentration, U_{TA} is the urine titratable acid concentration, and U_{HCO3} is the urinary bicarbonate concentration. Thus, the components of tubular acid-base homeostasis can be thought of as bicarbonate reclamation (fundamentally happening in the proximal convoluted tubule), as well as ammonium and titratable acid excretion (primarily taking place in the distal nephron) [5, 21].

HCO_3^- Reabsorption

One of the main functions of the kidneys is the excretion of waste products. To this end, our kidneys maintain a high glomerular filtration of about 150–180 l per day in a healthy adult. As bicarbonate is freely filtered in the glomerulus, approximately 4000 mmol/day of it must be reabsorbed by the tubules to prevent excessive loss of alkali and subsequent acidosis. Different transporters and enzymes in the proximal tubule work together to reabsorb approximately 80% of the filtered bicarbonate in this segment [22].

The mechanisms for proximal reabsorption are shown in Fig. 1. The two main transport processes in this segment are H^+ secretion at the luminal membrane, via a specific Na^+/H^+ exchanger (NHE3/SLC9A3) and HCO_3^- transport at the basolateral membrane via a Na^+/HCO_3^- cotransporter (NBC1/SLC4A4), while both processes are supported by the activity of two different isoforms of the enzyme carbonic anhydrase (CA II and IV) [23].

NHE3 provides roughly 65% of apical proton secretion [24]. Transport is electroneutral, driven by a lumen-to-cell Na^+ gradient (maintained by the basolateral Na^+/K^+ ATPase), stimulated by intracellular acidosis, and inhibited by high concentrations of amiloride [23]. The remaining third of the proton secretory rate is provided by a H^+-ATPase located in the luminal membrane which derives its energy directly from ATP [24].

Once the hydrogen ion is in the lumen of the proximal convoluted tubule, it combines with bicarbonate to form carbonic acid (H_2CO_3), which will then form carbon dioxide and water. The enzyme carbonic anhydrase is critical for catalyzing this process [25]. One isoform of this enzyme (CA IV) is located in the brush border membrane of the proximal tubule and serves to catalyze the hydrolysis of carbonic acid into H_2O and CO_2 (the latter diffusing through the apical membrane back to the cytosol) while another isoform of the enzyme (CA II) is cytosolic and catalyzes the reverse reaction, so that the resulting proton can be secreted into the lumen and bicarbonate reclaimed across the basolateral membrane [26].

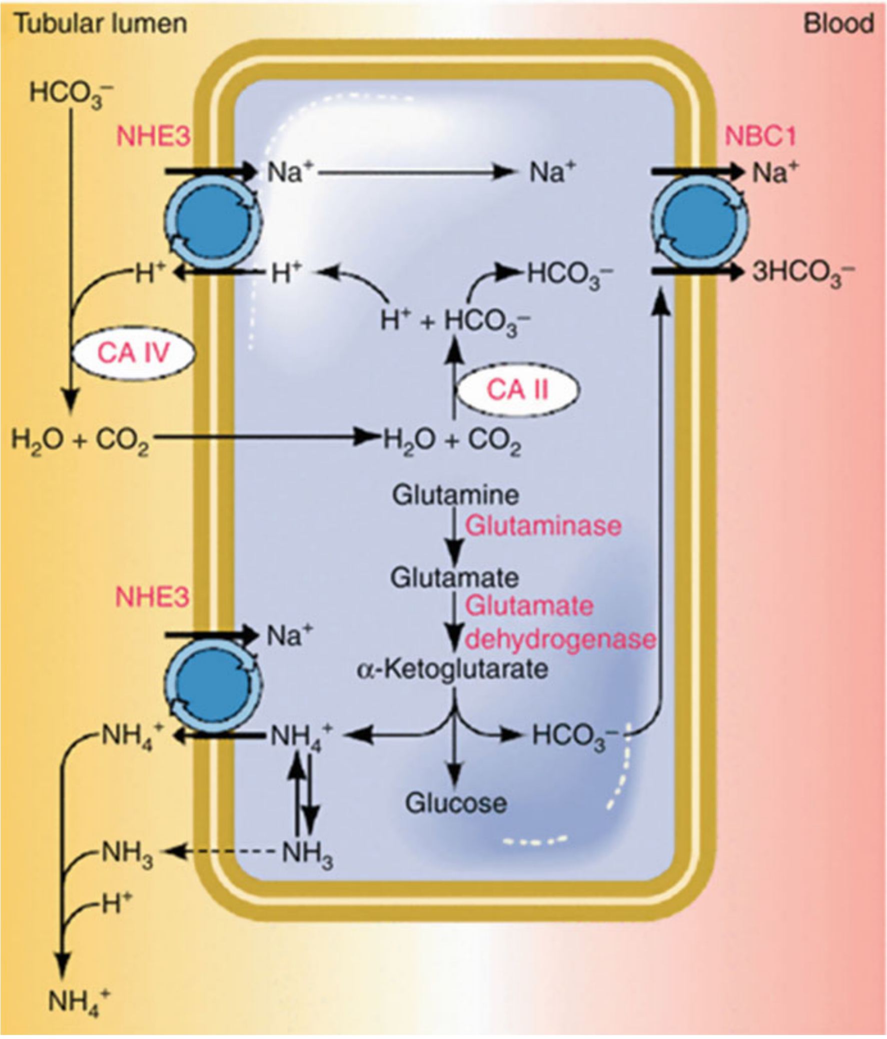
Tubular lumen
Blood
HCO_3^-
NHE3
Na^+
Na^+
NBC1
Na^+
H^+
H^+
HCO_3^-
$3HCO_3^-$
$H^+ + HCO_3^-$
CA IV
CA II
$H_2O + CO_2$
$H_2O + CO_2$
Glutamine
Glutaminase
Glutamate
Glutamate dehydrogenase
NHE3
Na^+
α-Ketoglutarate
NH_4^+
NH_4^+
HCO_3^-
Glucose
NH_3
NH_3
H^+
NH_4^+

Fig. 1 Model of bicarbonate reabsorption by a proximal tubule cell. The Na–K–ATPase located in the basolateral membrane generates and maintains the low intracellular sodium concentration. Protons are excreted into the tubule lumen by the sodium-proton exchanger (NHE3) where they combine with bicarbonate to form carbonic acid. In the presence of carbonic anhydrase IV (CAIV), the carbonic acid is hydrolyzed to water and carbon dioxide which enter the cell and recombine to form carbonic acid by the action of intracellular carbonic acid II (CAII). The carbonic acid ionizes into a proton which is then excreted into the lumen and bicarbonate which is transported by the sodium bicarbonate symporter (NBC1) into the bloodstream. (Reprinted with permission from Fry and Karet [157])

One of the particular characteristics of bicarbonate reabsorption within the proximal tubule is that its transport is saturable [27], which implies that in conditions of rising blood bicarbonate the filtered load of bicarbonate can potentially exceed the kidney's reabsorption rate and therefore bicarbonate will be lost in the urine. Studies in humans with normal proximal tubular function have provided evidence for such physiological titration curves [27].

In addition, about 20% of filtered bicarbonate is reabsorbed by passive back-diffusion along the paracellular pathway. Overall, proximal bicarbonate reabsorption is influenced by a number of factors, including luminal bicarbonate concentration and flow rate, extracellular fluid volume, peritubular bicarbonate concentration, and pCO_2, Cl^-, K^+, Ca^{2+}, phosphate, parathyroid hormone, glucocorticoids, α-adrenergic tone, and angiotensin II [28].

The thick ascending limb of Henle's loop reabsorbs about 15% of the filtered bicarbonate load by a mechanism similar to that present in the proximal tubule, i.e., through Na^+/H^+ apical exchange.

Ammonia Generation and Recycling

The generation of ammonia (NH_3) plays an essential role in the maintenance of the acid-base balance by the proximal tubule, and this is mainly the case due to its capacity to buffer hydrogen ions [29]. The importance of this becomes apparent, when considering the acid load in concentration terms: If the minimal urine pH is 5, this is equivalent to a H^+ concentration of 10 μmol/l. Thus, an acid load of 100 mmol unbuffered H^+ would require a minimal volume of urine of 10,000 liters for excretion!

The high pKa (~ 9.0) of the reaction $NH_3 + H^{+-} \rightarrow NH_4^+$ implies that within an average urinary pH of 7.0, virtually all of the ammonia (NH_3) in the urine will be present as ammonium (NH_4^+), hence limiting the amount of free hydrogen ions in the urine and making the ammonium excretion rate a quantitatively more important factor for the excretion of acid than urinary pH.

Ammonia is generated in the proximal tubule. The metabolism of glutamine provides two molecules of NH_3, secreted into the tubular lumen mainly as NH_4^+, and two of HCO_3^- which are subsequently transported to the basolateral membrane. Half of the produced ammonia is excreted in the urine, and the other half accesses the systemic circulation in order to produce urea in the liver (an event that requires consumption of HCO_3^-, and therefore the net effect on acid-base balance is neutral under physiological circumstances).

The proportional relationship between excreted and retained ammonia is modified by acidosis, and in consequence, urinary ammonia can transiently exceed ammoniagenesis [30]. The ability of the neonatal kidney to upregulate ammonium excretion is somewhat limited and can prolong the recovery phase of acidosis in infants. The upregulation of ammonium production and secretion serves as the principal means of correcting acidosis that is due to nonrenal causes. Indeed, the inability of the kidney to secrete acid as ammonium is a key feature of distal RTA.

Ammonia is electroneutral and can diffuse across membranes. In contrast, ammonium is a cation and thus membrane impermeable, but the ionic radius is similar to potassium, and therefore ammonium can substitute for potassium on many potassium transporters. The difference in membrane permeability between ammonia and ammonium causes a phenomenon called "ammonia trapping," where binding of ammonia to protons in the tubular lumen "traps" it there.

In the proximal tubule, NH_4^+ is secreted into the tubular lumen by NHE3/SLC9A3 (sodium hydrogen exchanger 3) and also by ammonia trapping. In the thick ascending limb of the loop of Henle, NH_4^+ is then reabsorbed predominantly by NKCC2/SLC12A1, diffusing across the basolateral membrane as NH_3 and also transported via a sodium and NH_4^+ exchanger NH4 [31]. An overview of ammonia transport is given in Fig. 2.

Finally, in the collecting duct, ammonia is trapped again in the tubular lumen by proton secretion [32]. The transport of NH_3 through the basolateral membrane in this segment is further facilitated by the ammonia-specific transporters Rhesus glycoproteins Rhbg and Rhcg and also the Na/K-ATPase. Cytosolic NH_3 is transported across the apical membrane by a combination of Rhcg and diffusion, while apical H^+ secretion involves both H^+-ATPase and H^+-K^+-ATPase [33].

Distal Tubular H^+ Secretion

In the distal nephron segments, urinary acidification is achieved via three related processes [23]:

1. Reabsorption of the small fraction of filtered HCO_3 that escaped previous segments (~10–20%)

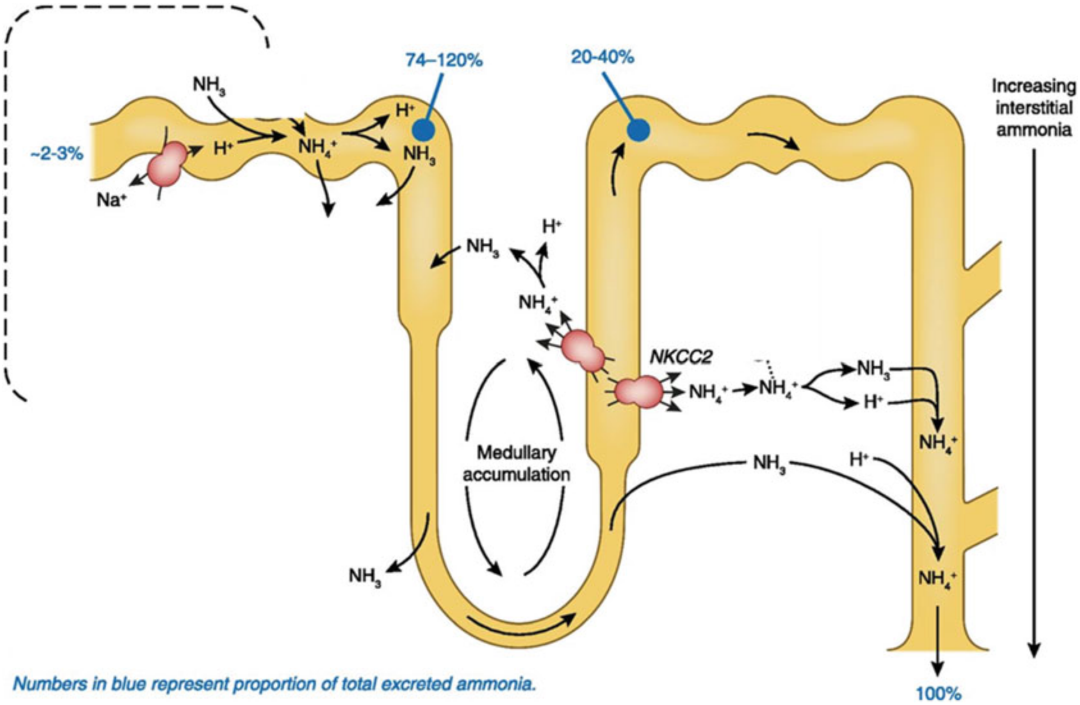

Fig. 2 Overview of renal ammonia transport. Renal ammoniagenesis occurs primarily in the proximal tubule from glutamine metabolism, forming ammonium and bicarbonate. There is apical NH_4^+ secretion involving NHE3 and parallel H^+ and NH_3 transport. Ammonia reabsorption in the thick ascending limb occurs via apical NKCC2-mediated uptake and results in medullary ammonia accumulation. Ammonia is secreted in the collecting duct via parallel H^+ and NH_3 secretion. Ammonia (NH_3) is "trapped" in the tubular lumen by binding to H^+, forming ammonium (NH_4^+). The numbers in blue represent the proportion of total excreted ammonia. (Modified from [158])

2. Titration of divalent basic phosphate (HPO_4^{2-}), which is converted to the monovalent acid form ($H_2PO_4^-$)
3. Luminal accumulation of ammonia (NH_3), which buffers H^+ to form nondiffusible ammonium (NH_4^+)

The collecting duct contains three different epithelial cell types: the principal cells and the intercalated α- and β-cells. Secretion of protons occurs via a vacuolar H^+-ATPase expressed in the apical membrane of α-intercalated cells [34, 35]. Bicarbonate generated in this process is exchanged for chloride ions at the opposite pole of the cell (basolateral membrane) via the anion exchanger AE1/SLC4A1 [36]. Chloride can then exit the α-intercalated cell through the apical membrane via the potassium chloride cotransporter (KCC) or the chloride channel, CLCNKB [37, 38]. Lastly, carbonic anhydrase (CA) isoenzymes II (cytosolic) and IV (luminal side of the plasmatic membrane) play a critical role in catalyzing the production of proton and bicarbonate ions intracellularly, but also the breaking down of carbonic acid into water and CO_2 in the tubular lumen, with CO_2 diffusing back into the cells allowing the regeneration of bicarbonate [25].

The β-intercalated cells are characterized by an inverted polarity of transporters compared to the α- cells and so extrude bicarbonate into the tubular lumen rather than protons. The apical Cl^-/HCO_3^-exchanger, Pendrin (SLC26A4), is expressed in the apical membrane; HCO_3^- is secreted into the luminal space while both ATPases, the H^+-pump and the H^+/K^+ exchanger, are present in the basolateral membrane. Thus, β-intercalated cell activity is important for correcting metabolic alkalosis.

The principal cells of the collecting duct are responsible for the reabsorption of sodium and the secretion of potassium and do not directly secrete protons into the tubular fluid. Their activity however influences the rate of acid secretion indirectly, by affecting the electrical potential difference across the epithelium. This is the reason why factors (disease or drugs) with primary effect on sodium/potassium transport in the collecting duct can disturb acid-base balance [39]. Distal urinary acidification is influenced by multiple factors, including blood pH and pCO_2, distal sodium transport and transepithelial potential difference, aldosterone, and potassium [23].

Besides ammonia, urinary protons can also be buffered by titratable acids, which include phosphate, sulfate, and other anions. From these, phosphate is quantitively the most important. Inorganic phosphate can be found in the blood as different ionic species (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}), with $H_2PO_4^-$ and HPO_4^{2-} being the most abundant at physiological values of pH. The pK for the equilibrium between $H_2PO_4^-$ and HPO_4^{2-} is ~6.8. Therefore, at an average blood pH of 7.4, the $[H_2PO_4^-]/[HPO_4^{2-}]$ ratio is approximately 4:1. While urine is flowing through the collecting duct where the pH is lower, HPO_4^{2-} tends to accept protons, becoming $H_2PO_4^-$, and therefore participates in acid buffering [39].

Proximal Renal Tubular Acidosis

Proximal RTA (pRTA) refers to the impaired ability of the proximal tubule to reabsorb bicarbonate and generate ammonia.

Pathophysiology

Bicarbonate reabsorption in the proximal tubule is a saturable process (see above). The threshold for bicarbonate reabsorption will be the main determinant of the blood bicarbonate concentration and is normally around 24–26 mmol/L. If the bicarbonate concentration in blood exceeds this threshold, transport will be saturated, and bicarbonate will start to be excreted in the urine, bringing the blood levels down until it matches the threshold, and then all of the filtered bicarbonate will be reabsorbed again.

pRTA is mainly characterized by a reduced threshold for the reabsorption of bicarbonate [40]. Typically patients with pRTA tend to have thresholds around 14–18 mEq/L [41]. It is this reduction in the capacity for reabsorption of bicarbonate that makes the treatment of patients with pRTA particularly challenging: As the patient is treated with bicarbonate and the blood bicarbonate rises, bicarbonaturia will increase, limiting the increase in the blood bicarbonate concentration [42].

Hypokalemia is a common finding in patients with pRTA and likely relates to impaired proximal potassium reabsorption (in generalized proximal dysfunction), and also from volume depletion and increased serum aldosterone concentration, as NHE3-mediated bicarbonate recovery is also a key pathway for proximal sodium reabsorption [43].

Although treating these patients can be particularly challenging, their acidemia is only moderate. Having a stable tubular reabsorption threshold means that all filtered bicarbonate can be reabsorbed once the serum levels match the threshold [44, 45]. Therefore, the serum bicarbonate concentration will not fall below this threshold, which is usually about 14–16 mmol/L. Notably, distal tubular acidification is intact, and the urinary pH can fall below 5.3 in proximal RTA. Thus, when the serum bicarbonate is below the tubular reabsorption threshold, the distal tubule can excrete the daily acid load and maintain acid-base balance. This is reflected in normal calcium excretion rates and the absence of rickets or osteomalacia in patients with isolated pRTA, although short stature is a common finding [44].

Renal Fanconi Syndrome

Besides reabsorbing the bulk of filtered water and solutes, the proximal tubule is also the sole nephron segment responsible for the reabsorption of low-molecular weight proteins (LMWP), glucose, amino acids, and phosphate. A generalized failure of the proximal tubule is referred to as renal Fanconi syndrome (RFS) and is characterized by a typical biochemical “fingerprint,” which includes hypokalemic metabolic acidosis with hypophosphatemia and elevated excretion of glucose, phosphate, LMWP, and aminoacids in the urine.

PRTA almost invariably occurs in the context of RFS, which is discussed in Chap. “Fanconi Syndrome. Renal Tubular Disorders.” In contrast, isolated pRTA is exceedingly rare.

Etiology

Hereditary Isolated pRTA

Inherited pRTA has been described with autosomal dominant and autosomal recessive patterns of inheritance.

Autosomal recessive inheritance in isolated pRTA has been linked to variants in *SLC4A4*, encoding the basolateral Na^+/HCO_3^- exchanger [46, 47]. Besides pRTA, these patients have prominent eye abnormalities, such as band keratopathy. So far, only very few cases have been described in the literature, reflecting the extreme rarity of this disorder [48–50].

PRTA with autosomal dominant inheritance pattern also appears to be very rare and has so far been reported in only two families [44, 46]. Yet, despite the first family being reported already in 1977, attempts at identification of the underlying genetic problem have so far been unsuccessful.

Diagnosis

The formal diagnosis of pRTA requires the demonstration of urinary bicarbonate wastage with systemic acidosis [51]. Traditionally, a fractional bicarbonate excretion of $\geq 15\%$ is said to be needed to establish the diagnosis of pRTA [52]. Given the capacity of the distal nephron to reabsorb bicarbonate, however, even mild bicarbonate wastage (fractional excretion $\sim 5\%$) could be sufficient for the diagnosis. The most definitive way to diagnose pRTA is to assess bicarbonate excretion when blood bicarbonate is increased by administration of sodium bicarbonate (HCO_3^-titration test). When pRTA is present, a marked increase in urinary bicarbonate excretion and urine pH occurs as blood bicarbonate rises above the renal threshold, whereas bicarbonate excretion decreases and the urine pH falls when blood bicarbonate is reduced [51].

Such formal tests for bicarbonate excretion are however rarely performed in clinical practice, and attention to a few clinical features is usually sufficient to distinguish pRTA from the distal form [53, 54]:

1. Concomitant presence of glycosuria, aminoaciduria, hyperphosphaturia, and LMWP excretion suggesting RFS or, alternatively, of the typical eye findings suggesting SLC4A4-related disease.
2. Intact ability to lower urine pH <5.3 when the blood bicarbonate is lower than the renal threshold for bicarbonate reabsorption (i.e., without bicarbonate supplementation).
3. Urinary anion gap is normal/negative (while in distal RTA it is consistently positive).
4. Skeletal abnormalities and rickets are less common compared to distal RTA.
5. Nephrocalcinosis and urolithiasis are rare.

Treatment

Patients with inherited and acquired pRTA are usually given alkali supplements [42]. Particularly in children, alkali replacement may be helpful to support growth [55]. Unfortunately, as soon as blood bicarbonate levels exceed the threshold of reabsorption in the proximal tubule, the excess bicarbonate is lost in the urine. Consequently, the higher the dose, the more is lost in the urine, and prescribed doses as high as 15 mEq/kg/day have been reported [51]. Bicarbonate loss in the urine is most pronounced if supplementation is given in infrequent large doses: After each dose, blood bicarbonate will briefly rise, leading to exaggerated losses until the blood concentration has decreased again to the threshold level. For this reason, supplementation should be sustained over as long a time as possible to avoid such brief peaks in serum levels with consequent large increases in urinary losses.

Some authors also recommend the concomitant use of a thiazide diuretic in order to reduce bicarbonate losses. The principle behind this idea is to enhance proximal tubule and loop bicarbonate reabsorption by inducing a contraction of the extracellular volume [56]. However, the addition of a thiazide can aggravate hypokalemia by promoting potassium excretion. Moreover, the loss of sodium bicarbonate in itself causes volume contraction, further compounded by thiazides, and for these reasons this therapy should be carefully considered and monitored.

Distal Renal Tubular Acidosis (Type I)

Distal RTA (dRTA) refers to the impaired ability of the distal tubule to secrete protons.

Pathophysiology

As initially defined in 1967 [57], distal renal tubular acidosis describes the impaired ability of the kidney to maximally decrease the urine pH under conditions of systemic acidosis. The molecular mechanisms (Fig. 3) underlying this process were discovered at the end of the twentieth century by a combination of physiologic studies, molecular cloning, and genetic investigations in inherited forms of dRTA [58]. This secretion should match the daily acid load to maintain overall acid-base balance. A common western diet generates a daily acid load of 1 mmol/kg of body weight [18], and children typically produce an extra 1–2 mmol/kg of body weight for bone mineralization (formation of hydroxyapatite), generating approximately 2–3 mmol/kg/day that the kidneys need to excrete [19, 20].

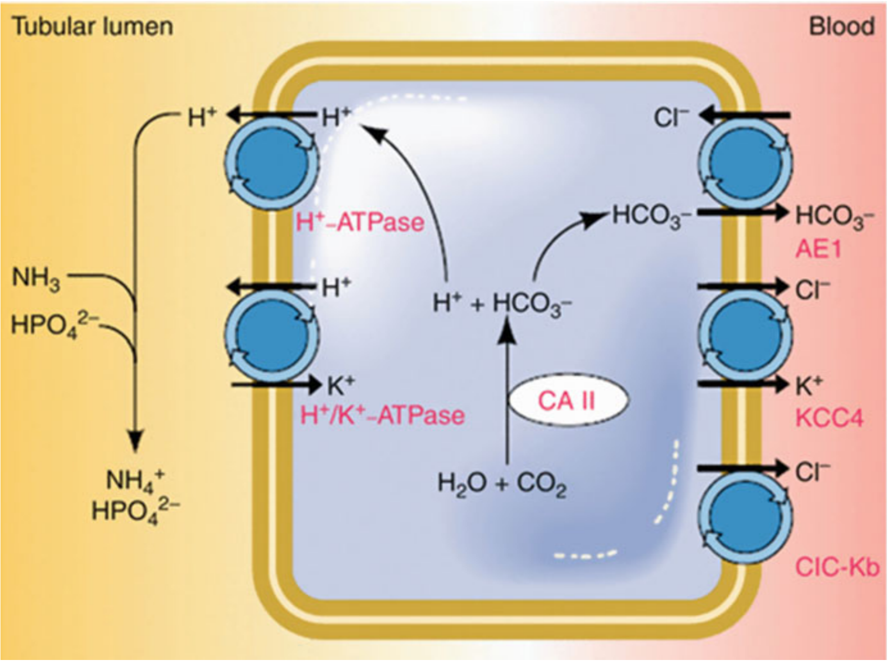

Fig. 3 Model of acid excretion in an alpha-intercalated cell in the distal nephrons. Protons are excreted into the tubule lumen by the proton ATPase and are buffered by ammonia or titratable acid (mostly phosphate). Inside the cell, carbonic anhydrase II (CAII) provides the protons and bicarbonate through the hydration of carbon dioxide to form carbonic acid. Bicarbonate is excreted into the bloodstream by the action of the chloride bicarbonate exchanger (AE1) on the basolateral membrane. Chloride homeostasis is maintained by the potassium chloride cotransporter (KCC4) and the chloride channel (ClC-Kb). (Reprinted with permission from Fry and Karet [157])

Pathophysiological Consequences

The distal inability to excrete excess body acid will cause a progressive acidosis with increase of the base deficit. Pathophysiologic consequences include nausea, vomiting, fatigue, tachypnea, mineral bone disease, hypercalciuria, salt wasting, hypocitraturia, nephrocalcinosis/urolithiasis, and also proximal tubular dysfunction [59–62].

Bone Disease

At the extracellular level, bicarbonate is consumed due to its buffering action, and once depleted, other buffers start to take over. The most abundant is phosphate, which is stored predominantly in the skeleton in the form of hydroxyapatite [$Ca_5(PO_4)3$ (OH)]. Hydroxyapatite dissolves in an acidic environment, releasing not only hydroxyl ions (OH^-), but also calcium which subsequently must be excreted in the urine, leading to hypercalciuria and calcification (nephrocalcinosis/urolithiasis). Thus, patients with untreated dRTA have a negative calcium balance [1].

In children, the clinical consequence of this is the development of rickets, while in adults it is osteomalacia [7]. The exact physiopathological mechanisms linking mineral bone disease with systemic acidosis are yet to be elucidated. In vitro studies using rat cells suggest that acidosis does not directly affect the composition of the mineral osteoid but stimulates the resorptive activity of the osteoclasts by directly activating the cells [63] and also inhibiting the synthetic function of osteoblasts [64]. In contrast, data from bone biopsies in patients with dRTA showed suppressed activity of both osteoblast and osteoclasts with low turnover bone disease and reduced mineral density [65]. Importantly, these findings are reversible with treatment of the acidosis [66].

Hypercalciuria and Nephrocalcinosis

Not only hypercalciuria contributes to the risk of nephrocalcinosis, but also the increased proximal reabsorption of citrate with subsequent hypocitraturia. Citrate is the most important and abundant organic urinary base and also the most important chelator for calcium in the urine. It is an antilithogenic agent by preventing calcium binding to oxalate or phosphate with consequent precipitation. The reabsorption of citrate happens exclusively in the proximal tubule through a specific cotransporter (sodium-dicarboxylic acids) NaDC1/SLC13A2 which shows increased expression in the apical membrane during systemic acidosis [67, 68]. Once citrate is taken up by the proximal tubular epithelial cell, it is metabolized by the mitochondria through the citric acid cycle, generating CO_2 and H_2O while consuming two or three protons (H^+). This suggests that a normally functioning proximal tubule is needed to develop hypocitraturia and may explain why nephrocalcinosis and hypocitraturia are typical in distal RTA and not in proximal RTA or the renal Fanconi syndrome.

Nephrocalcinosis in itself can have a negative impact on patients' prognosis with studies suggesting that it may accelerate loss of kidney function by triggering a local inflammatory response [69, 70]. It also may impair the urinary concentration ability, presumably due to interstitial calcification [71]. Nephrocalcinosis has also been associated with erythrocytosis, presumably reflecting impaired interstitial perfusion [72, 73]. Importantly, nephrocalcinosis in itself can be associated with dRTA, suggesting that it can be a cause as well as a consequence of dRTA.

Urolithiasis shares the same pathophysiological mechanisms with nephrocalcinosis and as such is common in patients with dRTA, with selected series from tertiary centers showing a prevalence as high as 77% in primary dRTA, and this seems to be also true for secondary forms [74, 75]. The combination of hypocitraturia, hypercalciuria, and a high urinary pH creates a favorable environment for calcium-phosphate precipitation, and brushite is the predominant mineral found in urinary stones from patients with dRTA. However, other stone compositions are also seen, including struvite, calcium oxalate, purines, and mixed stones perhaps reflecting the particularities of each individual and their respective stone formation risk: recurrent urinary tract infections, the degree of urinary acidification impairment, diet, and other comorbidities [74].

Hypokalemia

Hypokalemia, although variable, is another classical finding in patients with dRTA [76, 77]. The exact mechanisms involved in potassium wasting have not been fully elucidated but likely include a combination of different factors. Failure of distal acid secretion leads to an electrochemical gradient that favors potassium secretion. Renal potassium wasting through compensatory overactivity of the H^+/K^+-ATPase in the collecting tubule has also been proposed [76]. In addition, impaired urinary concentrating ability and subsequent increased urinary flow rate activate K-secretion via BK-channels [31]. Finally, hyperaldosteronism [77] related to volume contraction has been documented and may contribute to further urinary potassium wasting.

Hypokalemia can be very severe, causing rhabdomyolysis [78] and muscle paralysis, which can be one of the presenting signs of dRTA [79]. In a report describing 14 patients with hypokalemic rhabdomyolysis, 7 patients had an underlying diagnosis of dRTA [78].

Salt Wasting

Not only potassium wasting is a feature of dRTA, but sodium reabsorption can be also impaired, likely due to impaired exchange with protons. But also acidosis per se may cause decreased sodium reabsorption as the abnormally low pH can affect the normal functioning of several transporters [80].

Impaired Ammonia Excretion

Ammonium excretion accounts for the majority of basal bicarbonate generation, and changes in its excretion rate are the primary response to acid-base disorders (see also “ammonia generation” above) [31].

The failure to “trap” ammonia in the tubular lumen via H^+ secretion is associated with impaired ammonia excretion [80]. Indeed, hyperammonemia has been reported as one of the presenting features in patients with distal renal tubular acidosis particularly during severe acidotic episodes [81]. It may be more common than reported, as ammonia is not routinely measured in clinical practice.

From a small selected Japanese cohort of 11 patients with pathogenic variants in subunits of the proton pump, approximately one-third [4] were found to have raised levels of ammonia in the blood at presentation [82]. However, to what degree these raised ammonia levels reflect insufficient excretion versus an acidosis-associated defect in metabolization is unclear. The fact that the levels are only reported to be raised during severe acidosis argues for the latter, as impaired ammonia trapping is a permanent feature of dRTA.

Etiology/Genetics

So far, five different genes have been found to cause inherited distal RTA (see Table 1).

Very recently, a single patient with an unusual phenotype of acidosis and end-stage kidney disease with death in infancy was described with a homozygous variant in a nonpreviously disease associated subunit (*ATP6V1C2*) of the H^+-ATPase pump [88]. However, until further patients are identified, *ATP6V1C2* remains a gene of uncertain significance with respect to distal renal tubular acidosis [89].

Table 1 Genes associated with dRTA listed are currently recognized genes associated with dRTA. AD, autosomal dominant; AR, autosomal recessive; SND, sensorineural deafness; AI, amelogenesis imperfecta; N/A, not available

Gene	Function	Phenotype MIM	Inheritance	Extrarenal features	References
SLC4A1	Anion exchanger (AE1)	179,800	AD, AR	Occasionally hemoglobinopathy	[83]
ATP6V1B1	V-type H^+ ATPase subunit B1	267,300	AR	SND	[84]
ATP6V0A4	V-type H^+ ATPase subunit a4	602,722	AR	+/−SND	[85]
FOXI1	Transcription factor FOXI1	N/A	AR	SND	[86]
WDR72	WD repeat-containing protein 72	N/A	AR	AI	[87]

Genotype-Phenotype Correlation

Sensorineural Deafness

Additional clinical features can be helpful to pinpoint the underlying genetic cause. As the vacuolar proton pump is also expressed in the inner ear, patients with dRTA associated with ATP6V1B1, FOXI1, and to a lesser degree, ATP6V0A4 typically have sensorineural hearing loss. FOXI1 is a transcription factor controlling expression of several genes involved in acid secretion, including subunits of the proton pump, and as such it does share a similar phenotype.

Hemoglobinopathy

Patients with AR mutations in anion exchanger 1, typically from Southeast Asian ethnic background, can have concomitant ovalocytosis or spherocytosis (see section "SLC4A1," below).

Amelogenesis Imperfecta

WDR72 was initially described as a disease gene for amelogenesis imperfecta, a congenital enamel defect [90]. Indeed, the presenting symptom in some patients with *WDR72*-associated dRTA was amelogenesis imperfecta, with dRTA only noted by "reverse phenotyping," i.e., further investigations prompted by the genetic result.

Molecular Aspects of Distal Renal Tubular Acidosis

SLC4A1

Pathogenic variants in *SLC4A1* affect the structure and/or function of the anion exchanger (AE1), a chloride/bicarbonate exchanger (Fig. 3) located in the basolateral membrane of the α-intercalated cells of the collecting duct and responsible for the transport of bicarbonate from the intracellular space to the systemic circulation. Retention of bicarbonate in the cytosol will cause an increase in the pH and therefore impairs the ability of the α-intercalated cell to secrete protons to the tubular lumen.

A different isoform of AE1, including an extended N-terminus, is expressed in the membrane of red blood cells where it was initially discovered and named "band 3 protein" [83, 91]. AE1 does not only control intracellular pH but also performs an important role in stabilizing the membrane structure of the erythrocytes.

AE1 dimers form a tetrameric macrocomplex between itself, the Rh complex, glycophorin A (GPA), and ankyrin, which links the membrane with the cell cytoskeleton [92]. Dysfunctional AE1 cannot adequately exchange anions and may induce a monovalent cation leak from the erythrocyte [93]. Carriers of specific heterozygous variants in *SLC4A1* have a mild hemolytic anemia due to destabilization of the erythrocyte membrane known as hereditary spherocytosis or Southeast Asian ovalocytosis (SAO) [94].

Most patients that carry pathogenic variants in *SLC4A1* have either a renal or a hematological phenotype, and there are different reasons:

1. Variants in the N-terminal fragment of the transmembrane protein not transcribed in the kidney will only affect red cell function.
2. Variants affecting intracellular sorting to the basolateral membrane will only affect kidney function, as the red cell is not polarized [95] and expresses chaperone proteins, such as Glycophorin A [96].
3. Variants causing distal renal tubular acidosis typically have a dominant negative effect, such as mistargeting or retention of the dimeric AE1 in the ER, whereas variants causing haploinsufficiency are associated with isolated red cell problems [96].

Some patients can develop distal renal tubular acidosis and chronic hemolysis [97]. This can occur in compound heterozygote individuals with an SAO and a distal renal tubular acidosis variant.

The erythrocyte cation leak from SAO variants has been linked to protection from Malaria infection, which would explain why such pathogenic variants are more prevalent in tropical areas [98].

SLC4A1-associated dRTA is typically inherited in an autosomal dominant fashion due to the dominant negative effect of the identified variants. However, *SLC4A1* variants associated with loss-of-function cause autosomal recessive dRTA.

ATP6V1B1 and *ATP6V0A4*

ATP6V1B1 and *ATP6V0A4* (formerly annotated as *ATP6N1B*) encode subunits of the vacuolar H^+-ATPase, located in the apical membrane of the α-intercalated cells (Fig. 3). This H^+-ATPase has two domains: a cytoplasmic or V1 domain, which hydrolyzes ATP and a transmembrane or V0 domain facilitating the actual transmembrane transport of protons, either to intracellular compartments or to the extracellular space [58]. Intracellularly, this vacuolar ATPase performs different tasks such as receptor-mediated endocytosis, membrane trafficking, prohormone synthesis, protein degradation, and neurotransmitter uptake, while it can also facilitate entry of various viruses and toxins. In the plasma membrane, they are involved in renal H^+ secretion, bone resorption, and sperm maturation [58].

V1 is the larger domain formed by eight different subunits (A-H) while V0 contains six subunits (a, d, e, c, c′ and c″) [99]. Isoforms B1 and a4 are expressed not only in the kidney but also in other tissues such as inner ear and epididymis, which explains why patients with pathogenic variants in these genes can have hearing loss. Despite acidification being a key factor for sperm cell conservation in the epididymis, male infertility has so far only been documented in the mouse model for *FOXI1* (see below) [100, 101].

ATP6V1B1 was the first recessive disease gene identified in dRTA, by investigating a cohort with concomitant bilateral, progressive, and early onset sensorineural hearing loss (SNHL) [84]. Specific radiological studies also revealed abnormally enlarged vestibular aqueducts; vertigo has been also described but appears to be rare [102, 103]. Subsequent investigations in individuals with dRTA without SNHL helped identify a second gene, *ATP6V1A4*, encoding a subunit of the V0 domain [85]. This cohort had a high prevalence of nephrocalcinosis and rickets with a very early age at presentation, and all had normal audiometry, suggesting a new subtype of dRTA without SNHL. Two years later, a subsequent study provided evidence for patients with mutations in *ATP6V0A4* also having hearing impairment albeit with a tendency to later onset, up to 33 years old [104].

FOXI1

FOXI1 encodes a forkhead transcription factor crucial in the expression of different proteins in acid-secreting epithelia, including: AE1, AE4, and the V-ATPase subunits B1, a4, A, and E2 [105]. Biallelic pathogenic variants in *FOXI1* are linked to a severe phenotype of dRTA with early and profound SNHL [86]. This phenotype had been previously reported in Foxi1 knockout mice, which also have male infertility [106]. Whether this particular phenotype will be relevant in humans is still unknown as follow-up data for the only male individual reported so far are limited to his childhood.

WDR72

WDR72 had been previously associated with amelogenesis imperfecta (AI) [90]. Later, recessive variants in *WDR72* were identified in two families affected by dRTA and AI [87], and subsequently further families were reported [107, 108]. The exact mechanisms by which *WDR72* variants cause distal renal tubular acidosis remain unclear at present. WDR72 has been implicated in intracellular vesicle trafficking [87]. Why some of these patients manifest either AI, dRTA, or both is also unclear. However, “reverse phenotyping” in one report showed that all individuals with dRTA and variants in *WDR72* also had AI [107]. Similarly, the late onset or absence of overt acidosis in some individuals with AI and *WDR72* variants may reflect that the dRTA phenotype can be mild or “incomplete” (see below) [108].

Other Genes

In most larger series, causative variants are identified in about 60–80% of patients with a clinical diagnosis of primary dRTA [16, 109], suggesting that further disease genes are yet to be identified.

Secondary dRTA

Secondary dRTA is typically associated with autoimmune disorders, especially Sjögren syndrome [110]. A list of reported causes is provided in Table 2.

Incomplete Distal Renal Tubular Acidosis

Incomplete dRTA refers to a disorder characterized by an impaired renal tubular acidification

Table 2 Conditions associated with secondary dRTA. Shown are conditions that are associated with dRTA and the presumed mechanisms. Urinary tract obstruction and hyponatriuretic states, as well as drugs blocking the epithelial sodium channel ENaC in the collecting duct (e.g., amiloride, trimethoprim) are typically associated with hyperkalemic or type 4 dRTA

Etiology	Examples	Presumed mechanism
Autoimmune disorders	Sjögren syndrome Systemic lupus erythematosus, Rheumatoid arthritis, autoimmune thyroid disease, primary biliary cirrhosis	Autoimmune response to antigens in the intercalated cells
Toxins/drugs	Amphotericin B Lithium Toluene Amiloride Trimethoprim Pentamidine Vanadium Calcineurin inhibitors	Damage to collecting duct cells or blockage of specific transport molecules associated with acid secretion
Nephrocalcinosis/ hypercalciuria	Primary hyperparathyroidism Hypercalcemic hyperthyroidism Vitamin D intoxication, idiopathic hypercalciuria, familial hypomagnesemia-hypercalciuria with nephrocalcinosis	Calcium-mediated damage/dysregulation of tubular cells
Hemoglobinopathy	Sickle cell disease	Ischemic damage to tubular epithelial cells
Renal transplantation	Rejection	Inflammatory damage to tubular epithelial cells
Urinary tract obstruction	Posterior urethral valves, obstructive bladder stone	Pressure-related distal tubular dysfunction
Hyponatriuretic states	Liver cirrhosis Nephrotic syndrome Congestive heart failure	Insufficient distal sodium delivery

without overt systemic acidosis that is generally identified during investigations for recurrent urinary stone disease. It was first reported in 1959 by Wrong and Davies while studying urine acidification in a large cohort of 68 individuals with oral ammonium chloride administration [9]. Although in the initial report the three identified patients with this condition did have nephrocalcinosis but no urolithiasis, subsequent studies have been highlighting also the high prevalence of recurrent stone disease in these individuals [111, 112].

Accurate estimations of the prevalence of incomplete dRTA in the general population are not possible to be established for several reasons including the lack of overt metabolic acidosis in normal situations plus the need of an acidification test for confirmation, a test with many associated difficulties, including nausea, vomiting, and abdominal pain, among others [113]. However, there is available data published in selected population such as stone formers, where the prevalence varies from as little as 2% to as high as 19% [112, 114, 115].

It is not very clear why some patients despite a tubular acidification defect do not present with overt systemic acidosis, but possible explanations include sufficient buffering produced by the skeleton via bone resorption. Indeed mineral bone disease and growth failure are both common features of children with incomplete dRTA and alkali supplementation has been shown to reverse this effect in children [116]. In adults, incomplete dRTA has been associated with low bone mineral density and risk of fractures. Studies in groups of patients with low mineral bone density have reported a substantial prevalence of incomplete dRTA up to 20% [117, 118].

The causes of incomplete dRTA may be the same as for the complete form, and some clinicians consider incomplete and complete dRTA as part of a continuum, where the former is preceding the latter. Different case reports in children support this hypothesis, particularly in patients with dominant dRTA, where incomplete dRTA during early childhood may be followed by systemic acidosis in later years [4, 119]. In addition, incomplete dRTA has been associated with heterozygous variants in *ATP6V1B1* and ATP6V04 (and the corresponding heterozygous knock-out mice), perhaps denoting some degree of haploinsufficiency or a mild dominant negative effect [120, 121]. Incomplete dRTA has also been described in patients with medullary sponge kidney [122], Sjögren syndrome [123], nephrocalcinosis [124], and drug toxicity.

Patients with incomplete dRTA have a higher risk of stone formation/nephrocalcinosis and share a similar urinary metabolic profile (hypocitraturia and more alkaline urine) with complete dRTA. Stone composition is therefore also similar (>95% of carbonate apatite in occasions) [125], although hypercalciuria is only variably associated with incomplete dRTA [126].

As with the full form, the treatment of patients with incomplete dRTA and recurrent stone formation is alkali supplementation. Because of the low prevalence of the disease in the general population, large randomized control trials assessing treatment are lacking. Nevertheless, citrate supplementation has been shown to improve bone health in longitudinal studies, while alkali therapy in the pediatric population had a positive impact on height SDS in both complete and incomplete dRTA [116, 127].

Diagnosis

History and Examination

Patients with dRTA typically present with nonspecific symptoms, mainly failure to thrive. Occasionally, hypokalemia-related complications may predominate. Some may also have associated rickets. Subjects with dominant dRTA may present later in childhood or adolescence with urolithiasis [65]. Key to the diagnosis are blood and urine tests.

Biochemistry: Blood

The suspicion of a diagnosis of dRTA usually follows the finding of a normal anion gap/hyperchloremic metabolic acidosis and hypokalemia. This constellation of findings is typical for bicarbonate loss, and in the absence of a history or signs of enteric losses, a diagnosis of RTA must be considered.

Biochemistry: Urine

The next diagnostic step is then assessment of the urine to distinguish between proximal and distal RTA.

Urine pH

The first important urine data is the pH. In pRTA, distal acidification is intact, and once the blood bicarbonate level is below the threshold for reabsorption, bicarbonaturia resolves and urine pH can reach maximal acidity. Thus, in pRTA prior to bicarbonate supplementation, urine pH is typically <5.3. In contrast, in dRTA, urinary acidification is impaired and urine pH is >5.3.

There are a few potential problems with measurement of urine pH:

- If urine is exposed to air, CO_2 will diffuse from urine into air, thereby lowering the concentration of both HCO_3^- and H^+ in the urine, leading to an artificially elevated urine Ph.
- Urea-splitting organisms will increase the concentration of NH_3 in the urine, leading to an artificially elevated urine Ph.

Thus, urinary pH should be measured in freshly voided samples, or ones obtained under oil to prevent CO_2 diffusion, which can be challenging in non-toilet-trained patients, such as infants. And while a pH meter is the most accurate means of determining urine pH, pH strips or multistix dipped in the urine may be more practical, if they avoid potential delays from sending the urine to the lab. If pH strips are used, an upper pH limit for maximal acidification of 5.5 is commonly used.

Urinary Anion Gap

Protons in the urine are mostly buffered by NH_3 (ammonia) to form NH_4^+(ammonium). Consequently, in an acidotic patient with normal urinary acidification, the urine contains a substantial concentration of NH_4^+. The absence of urinary NH_4^+ thus indicates a defect in distal acidification. In clinical practice, few laboratories routinely perform urinary NH_4^+ measurement, but it can be indirectly assessed, via the urine anion gap (UAG).

The main ionic components of urine are Na^+, K^+, and Cl^-. In analogy to plasma, a UAG can be calculated: $UAG = Na^+ + K^+ - Cl^-$. However, in contrast to the plasma anion gap, the UAG is expected to be negative during acidosis, reflecting an excess of unmeasured cations in the urine (NH_4^+). For this reason, it can also be referred to as the "urine cation gap." In patients with dRTA, the concentration of NH_4^+ in the urine is low, because of the impaired ability of the kidney to excrete protons. Thus, in distal RTA, the cation gap is absent and the UAG is positive (reviewed in [128]). Calculation of the UAG can therefore be used as an indirect assessment of distal urinary acidification, with a positive UAG indicating dRTA. Obviously, the calculation of the UAG is only meaningful in patients with acidosis, as patients with an alkali load will physiologically have a positive UAG. It also should not be used in patients with advanced kidney disease [129]. Moreover, if the urine contains substantial concentrations of other unmeasured cations, such as ketoacids, salicylates, or penicillin, the UAG should be used cautiously [130].

Urinary pCO_2

The use of pCO_2 in the assessment of urinary acidification was first proposed in 1974 [131]. It is based on the rationale that urinary pCO_2 should increase during urinary acidification, because of proton secretion and carbanhydrase activity, generating H_2O and CO_2 [132, 133]. The same technical problem of CO_2 diffusion (discussed under "urine pH" above) applies to urine pCO_2 measurement, and in clinical practice, this test is rarely, if ever, used.

Stimulation Tests of Urinary Acidification

Patients suspected of having incomplete dRTA, i.e., without overt acidosis, will need special tests to assess their maximal urinary acidification. Acidification tests with either hydrochloric acid (HCl) or ammonium chloride (NH_4Cl) had been used for many decades but were complicated and required sometimes up to 5 days of study. The short NH_4Cl test proposed by Wrong and Davies constitutes an easier and more practical test that subsequently became the gold standard in diagnosis [7, 9]. However, many patients have difficulties tolerating NH_4Cl, and for this reason, alternative tests have been proposed.

The Short Ammonium Chloride Test

The initial protocol was based on a single and slow (about 1 h) oral administration of 100 mg/kg ammonium chloride. Tolerance to the test tends to be challenging due to early development of nausea and vomiting. Alternative doses of 75 mg/m^2 in infants diluted and administrated via nasogastric tube have been described [134]. The entire test takes about 6–8 h to be completed and includes a baseline serum sample, and a control 2–4 h post NH_4Cl is given to confirm that metabolic acidosis has been induced ($tCO_2 < 21.0$ or 18.0 mmol/L in infants) plus serial urines. Individuals with normal urinary acidification capacity can drop urine pH under 5.3 and generally between 4.6 and 5.0. Urinary ammonium, if measured, is expected to rise.

The Prolonged Ammonium Chloride Test

To facilitate better tolerance, a prolonged test using a lower dose (50 mg/kg/day), but repeated over 3 days, has been proposed [135].

Furosemide Testing

The furosemide test is based on the observation that the administration of loop diuretics like furosemide increases distal sodium delivery, as well as aldosterone secretion with consequent generation of a negative transmembrane voltage which will favor H^+ secretion into the tubular lumen [136–138].

The suggested furosemide dose is 1 mg/kg and administration either intravenous or orally with urine samples being collected during a period of 4 h. Urinary pH in normal individuals should drop below 5.3 whereas urine NH_4^+ and potassium excretion increase 2–3 times with respect to baseline values [138, 139].

Furosemide + Fludrocortisone Testing

The furosemide test was later modified to maximize stimulation by addition of exogenous mineralcorticoids [137]. A standard adult protocol uses furosemide (40 mg) and fludrocortisone (1 mg). Performance is better tolerated by the patients with no gastric irritation, and acidification capacity is equivalent to ammonium chloride test but significantly quicker [140]. In patients with recurrent urolithiasis, the F + F test has been reported to have a sensitivity of 85% and a specificity of 77%, compared to the short ammonium chloride test. However, the positive predictive value was only about 30%, whereas the negative predictive value was 98%, using the ammonium chloride loading test as a gold standard [141]. Thus, in adult stone-forming patients, a positive F+F test may need to be confirmed by a formal ammonium chloride-loading test.

Other Urine Abnormalities

Patients with dRTA occasionally have additional transient features of proximal tubular dysfunction, typically low-molecular weight proteinuria, aminoaciduria, and less commonly, phosphaturia [4, 43, 59–61]. In one series, this selective proximal tubular dysfunction was noted in 67% of dRTA patients at presentation [4]. The etiology of this proximal tubulopathy in dRTA is unclear but is commonly ascribed to the hypokalemic acidosis, as it resolves with treatment [60]. Interestingly, glycosuria has so far not been reported in this transient proximal tubular dysfunction in dRTA, and the presence of glucose in the urine would thus make a diagnosis of dRTA less likely [4].

Imaging

Medullary nephrocalcinosis is present in almost all patients [138]. Urolithiasis, although more prevalent in adulthood, can also present earlier [142].

Genetic Testing

Genetic testing should be offered to all patients with suspected inherited dRTA to confirm the diagnosis and facilitate genetic counseling. Early presentation, particularly in the first decade of life is very suggestive of an inherited form of distal renal tubular acidosis, although up to 10% of the patients can be diagnosed in adult life. Some clinical details can already provide clues as to the underlying gene: a positive family history with dominant inheritance suggests *SLC4A1*, whereas the presence of sensorineural hearing loss suggests one of the H^+ pump subunits or the transcription factor *FOXI1* as an underlying cause. Finally, the presence of enamel disorder suggests *WDR72*.

Treatment

The most important aspect of dRTA is that it is a treatable disease. Alkali supplementation, sufficient to match the daily acid load generated by the diet and bone metabolism, will normalize the biochemical abnormalities and most of the associated complications.

There are numerous different alkali supplementations available differing by:

1. Type of alkali (bicarbonate and/or citrate).
2. Type of salt (sodium, potassium, but also other alternatives as calcium or magnesium are available).
3. Formulation (liquid, tablets, immediate versus prolonged released).

In general, citrate forms are more frequently prescribed, presumably as at least in liquid formulations, and the alkali is usually more concentrated (2–3 mEq/ml) than in those containing bicarbonate (1 mEq/ml), thereby reducing the volume of medication needed. However, there is no solid evidence that citrate supplementation leads to a less lithogenic urine profile than bicarbonate [143, 144]. A large international study with 340 individuals diagnosed with primary distal renal tubular acidosis also showed no differences in metabolic control or long-term outcomes between patients taking sodium bicarbonate and sodium/potassium citrate [142]. And each form may cause problems with tolerability: Many patients find that citrate causes dyspepsia, whereas sodium bicarbonate may cause bloating and belching.

Recent trials of a new prolonged release formulation of potassium-bicarbonate and -citrate have shown a better gastrointestinal tolerance compared to standard treatment as well as improved metabolic control with only two administrations per day [145, 146]. However, real-world experience with this drug has yet to be reported.

In clinical practice, pragmatic considerations, such as local availability, affordability, and palatability, are probably most important to support effective treatment and compliance. If a Na^+-containing formulation is used, separate K^+ supplementation may be necessary to maintain normal plasma levels.

Dosage and frequency of alkali supplementation depend on multiple factors most notably the age of the patient: Infants have been prescribed doses up to 8–10 mEq/kg/day while older children and adults typically receive doses around 2–3 mEq/kg/day [4, 136]. Other factors include the underlying molecular basis, as well as diet (see below).

Infrequent administration of large doses of alkali supplementation may briefly increase blood bicarbonate levels above the threshold for proximal reabsorption, leading to loss of bicarbonate in the urine and potentially subsequent acidosis, once the remaining bicarbonate has been used up for buffering. To prevent this, administration should be several times a day. In infants, the total dose may be split up in small aliquots given with each feed, helping not only to maintain metabolic control but also minimizing side effects from unpleasant taste. In older children, less frequent dosing (3–4 times daily) is likely to help compliance and allow uninterrupted sleep. With the new extended release formulation, two daily administrations have been found to be safe and effective with potentially better compliance [146].

Mixed Proximal and Distal Renal Tubular Acidosis (Type III)

A combined pattern of proximal and distal features of RTA is observed in patients with impaired function in the enzyme carbonic anhydrase type II. This cytosolic enzyme is involved in the generation of carbonic acid from CO_2 and water in both proximal and distal segments of the nephron, facilitating bicarbonate reabsorption through the basolateral membrane to the interstitium and subsequently systemic circulation. Besides the kidney, CAII is also expressed in the brain and skeleton, and patients with CA II deficiency generally present with osteopetrosis, cerebral calcifications, as well as proximal and distal RTA. Early onset of hypokalemia, paroxysmal muscle weakness, moderate-to-severe neurodevelopment delay, and growth retardation are other associated features [12]. Characteristic biochemical findings besides the hypokalemic, hyperchloremic metabolic acidosis typical for all RTA reflect the combined proximal and distal defect: urinary bicarbonate wasting, failure to acidify urine below a pH of 5.5, a low urine-to-blood pCO2 difference in an alkaline urine, and decreased ammonium excretion [147].

CA type II is encoded by the *CA2* gene (OMIM #611492), and the associated disorder is inherited in an autosomal recessive inheritance pattern. Although more than 20 different pathogenic variants have been identified so far in individuals from different ethnic backgrounds, >70% of cases have been reported in the Maghreb [51, 148]. The splice variant (c.232 + 1G > A) identified repeatedly in Arabic patients is associated with a more severe phenotype compared to other cases in America and Europe associated with a missense variant (H107Y) [149].

Treatment of the acidosis is with alkali supplementation. The osteopetrosis can be treated with bone marrow transplantation.

Hyperkalemic Renal Tubular Acidosis (Type IV)

Type IV RTA is characterized by impaired aldosterone action: either due to deficiency or to resistance [39]. It is thus primarily a disorder of tubular salt handling and, because of the hyperkalemia, can be easily distinguished from any of the other presentations of RTA.

Pathophysiology

Aldosterone is involved in distal tubular acidification in several ways:

1. Stimulation of sodium reabsorption in the principal cells of the collecting duct. The resultant lumen negative electrical potential facilitates proton secretion and potassium secretion.
2. Upregulation of the expression of the H^+-ATPase and of the carbonic anhydrase in alpha-intercalated cells [150].
3. Stimulation of ammonia synthesis in the proximal tubule [151].

Aldosterone Deficiency

Aldosterone deficiency can be secondary to a generalized adrenal gland impairment, commonly known as Addison disease or to a specific inherited mineralcorticoid deficiency such as congenital adrenal hyperplasia (CAH). Isolated aldosterone synthase deficiency is a very rare condition but less severe than CAH as the glucocorticoid activity is generally preserved [152].

Aldosterone Resistance

Pseudohypoaldosteronism (PHA) refers to a condition where aldosterone secretion is unimpaired, but the kidney is unable to respond to it normally.

Pseudohypoaldosteronism Type 1 (PHA1)

Typically presents in early infancy. It is characterized by salt-wasting, hyperkalemia, and metabolic acidosis in the presence of markedly elevated plasma renin activity and aldosterone concentration. PHA1 does have two different inheritance patterns:

- An autosomal dominant form, due to heterozygous variants in the *NR3C2* gene, encoding the mineralocorticoid receptor. Clinical manifestations are limited to the kidney [153].
- An autosomal recessive form caused by biallelic loss-of-function variants in the genes encoding one of the three constitutive subunits (α- *SCNN1A*, β- *SCNN1B*, and γ- *SCNN1G*) of the epithelial Na^+ channel. Due to the expression pattern of this channel, clinical manifestations extend to different organs (kidney, large bowel, lung, and sweat and salivary glands) [154].

Pseudohypoaldosteronism Type 2 (PHA2 or Gordon Syndrome)

PHA2 is also characterized by hyperkalemia and metabolic acidosis, but in contrast to PHA1 by salt retention with consequent hypertension and suppressed plasma renin activity.

Four underlying genes have been identified: *WNK1*, WNK4, *KLHL3*, and *CUL3* [39]. Inheritance is typically autosomal dominant, except for *KLHL3* which can be associated with both dominant and recessive variants. Variants in *CUL3* are typically de novo and tend to be associated with extrarenal manifestation, especially growth failure [155].

Acquired Type IV RTA

Acquired aldosterone deficiency is rare in childhood and is typically associated with obstructive uropathy and interstitial kidney disease, such as pyelonephritis or tubulointerstitial nephritis. Medications causing type IV RTA obviously include those designed to interfere with aldosterone production or action, such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and potassium-sparing diuretics, such as the mineralocorticoid receptor blockers spironolactone and eplerenone or the ENaC blocker amiloride.

An acquired form of PHA2 can be seen in transplanted patients treated with calcineurin inhibitors [156].

Treatment

Treatment and prognosis depend on the underlying cause. In acquired forms, the underlying cause should be eliminated. Fludrocortisone is used in aldosterone deficiency. PHA1 is treated with sodium chloride and sodium bicarbonate supplementation and often with sodium-potassium exchange resins. PHA2 responds well to treatment with thiazides [59, 60].

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Aminoaciduria and Glycosuria in Children

39

Israel Zelikovic and Aude Servais

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Abstract

Hereditary tubular transport disorders that lead to abnormal aminoaciduria and glycosuria are uncommon but are of major biologic importance. Some of these diseases can be associated with significant morbidity. In the past two decades, remarkable progress has been made in our understanding of the molecular pathogenesis of hereditary aminoaciduria and glycosuria. Molecular genetics and molecular biology studies have led to the identification of numerous mutations resulting in aminoaciduria and glycosuria, have provided important insight into the defective molecular mechanisms underlying these disorders, and have greatly increased our understanding of the physiology of renal tubular reclamation of amino acids and glucose.

This chapter summarizes the general characteristics of proximal tubular transport of amino acids and glucose, discusses the

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specificity and classification of amino acid and glucose transport systems, reviews the molecular pathophysiology and genetic aspects of hereditary aminoaciduria and glycosuria, describes the clinical features of these tubulopathies, and summarizes their therapy. Special emphasis is given to cystinuria, lysinuric protein intolerance, Hartnup disease, iminoglycinuria, dicarboxylic aminoaciduria, familial renal glycosuria, and Fanconi-Bickel syndrome.

Further molecular studies of inherited proximal tubular transport disorders resulting in aminoaciduria and glycosuria may shed more light on the molecular pathophysiology of these diseases and may significantly improve our understanding of the mechanisms underlying renal handling of amino acids and glucose in health and disease. The identification of the molecular defects in these inherited tubulopathies may provide a basis for future design of targeted therapeutic interventions for molecular therapy of these complex disorders.

Keywords

Proximal tubule · Luminal membrane · Basolateral membrane · Membrane transport · Hereditary tubulopathies · Molecular pathophysiology · Heteromeric amino acid transporters · Gene mutation · Cystinuria · Urolithiasis

Synonyms

Grandparental involvement; Grandparental solicitude; Acidic amino acids; Anionic amino acids; Antiport; Basic (or Dibasic) amino acids; BBM - Brush border membrane; BBMV - Brush border membrane vesicles; Cationic amino acids; Convoluted proximal tubule; Counter transport; Early proximall tubule; Exchange transport; Late proximal tubule; LPI - Lysinuric protein intolerance; S1,S2 segments of the proximal tubule; S3 segment of the proximal tubule; SLC - Solute carrier family; Straight proximal tubule

Introduction

Only negligible amounts of amino acids and glucose are normally present in the final urine, reflecting very efficient reabsorption mechanisms for these organic solutes in the proximal tubule. Renal tubular transport defects or specific metabolic abnormalities result in excretion of significant quantities of amino acids or glucose in the urine. Although hereditary defects in renal tubular transport of most of these substances are uncommon, they are of major biologic importance. First, some of these membrane transport disorders (e.g., cystinuria, lysinuric protein intolerance, Hartnup disease, Fancoi-Bickel syndrome) are associated with significant morbidity. Second, the study of these disorders has provided much insight into the physiology of renal tubular reclamation of amino acids and glucose and into the specific metabolic pathways that control their reabsorption and has been crucial in understanding the genetics of tubular transport systems.

This chapter summarizes the general characteristics of renal tubular transport of amino acids and glucose, outlines the two main classifications used for amino acid transport systems (based on chemical properties/substrate specificity and sequence homology/structural features), and discusses the specific hereditary membrane transport disorders that result in abnormal aminoaciduria and glycosuria. Special emphasis is given to cystinuria, lysinuric protein intolerance, Hartnup disease, iminoglycinuria, dicarboxylic aminoaciduria, familial renal glycosuria, and Fanconi-Bickel syndrome, including molecular and genetic aspects of these diseases. This chapter does not discuss overflow aminoaciduria and glycosuria, which occur when the filtered load of these solutes exceeds the transport capacity of the renal tubule. The conditions leading to tubular overload include various inborn errors of amino acid metabolism and diabetes mellitus, which result in elevated plasma levels of amino acids and glucose, respectively. Fanconi syndrome, a proximal tubular disorder characterized by generalized aminoaciduria, and urinary hyperexcretion of glucose, bicarbonate, phosphate, and other solutes, is discussed in ► Chap. 37, “Cystinosis.”

Aminoaciduria

General Characteristics of Tubular Amino Acid Transport

Circulating free amino acids are derived from dietary proteins that are hydrolyzed and absorbed in the intestine, from intracellular catabolism of peptides, and from de novo synthesis within cells. More than 99% of the load of free amino acid filtered by the kidneys of humans and other mammals is reabsorbed in the renal tubule and returned to plasma [1–3]. Amino acid reabsorption occurs predominantly in the pars convoluta of the proximal tubule and, to a small extent, in the pars recta [1, 3]. Amino acids are reabsorbed primarily from tubular lumen by an active transport across the luminal membrane [1]. In studies using renal brush-border membrane vesicles (BBMV) from various animals to explore amino acid transport across this membrane, the rate of accumulation by vesicles and the magnitude of the overshoot, which indicates active concentrative transport, was greatly augmented by an external Na^+ gradient across the vesicle membrane [1]. Hence, it is widely accepted that uptake of most amino acids at the brush-border surface occurs by Na^+-amino acid cotransport driven by the electrochemical Na^+ gradient from the tubular lumen to the cell [1, 3] (Fig. 1). Maintenance of the Na^+ gradient is established by the Na^+-K^+-ATPase, which is located at the basolateral membrane and which transports Na^+ out of and K^+ into the cell. An additional driving force for amino acid transport across the luminal membrane is the H^+ gradient (luminal > intracellular) which drives uptake of proline [4] and glycine [5] by BBMV. Finally, cationic acids and cystine are reabsorbed across the luminal membrane in exchange for recycled neutral amino acids [2, 6] (Fig. 1).

Active amino acid transport across the brush-border membrane is followed by efflux, mainly via carrier-mediated, Na^+-independent, facilitated diffusion or exchange from the cell into the peritubular space across the basolateral membrane [2, 7] (Fig. 1). Thus, under normal conditions, net transepithelial movement of amino acids occurs from the tubular lumen to the peritubular space. However, net transepithelial flux of amino acids occurs from the combination amino acid transport in both directions, namely, lumen → interstitium and interstitium → lumen [2, 7, 8]. Indeed, the basolateral membrane harbors active Na^+-dependent and Na^+-independent transport and exchange systems mediating amino acid uptake in the tubular cell (Fig. 1), and diffusional backflux of amino acids from cell into the tubular lumen is a well-documented phenomenon [2, 7]. Interstitium → lumen oriented backflux through paracellular pathways also occurs. The sum of these vectoral fluxes determines the direction and the rate of transepithelial amino acid transport. These competing forces are important in understanding renal tubular amino acid transport, particularly in disease states and during maturation [8, 9, 10].

Na^+-amino acid symport across the luminal membrane is a carrier-mediated saturable process obeying Michaelis-Menten kinetics [1, 3]. The effectiveness of the active reabsorption process for a specific amino acid depends on the ratio $V_{max}:K_m$ [1]. A low V_{max} (decreased transport capacity) or a high K_m (diminished transporter-substrate affinity) for a given amino acid results in decreased reabsorption rate of this amino acid. Changes in efficiency or capacity of amino acid transport also have an important role in both neonatal aminoaciduria and hereditary aminoacidurias.

Microperfusion and micropuncture experiments and studies using BBMV for various amino acids [1] have identified two or more Na^+-linked transport systems with different kinetic characteristics. Having multiple transport systems for the same amino acid becomes very meaningful if reduced concentrations of filtered amino acid are presented to the proximal straight tubule. For example, in the case of glycine, two Na^+-dependent, active transport systems have been characterized along the luminal membrane of the isolated perfused proximal tubule [11]: a low-affinity, high-capacity system in the convoluted segment and a high-affinity, low-capacity system in the straight segment. The latter system absorbs less glycine against a greater concentration gradient and likely leads to lower luminal glycine concentrations than could be achieved in the proximal convoluted tubule [7, 11]. This axial

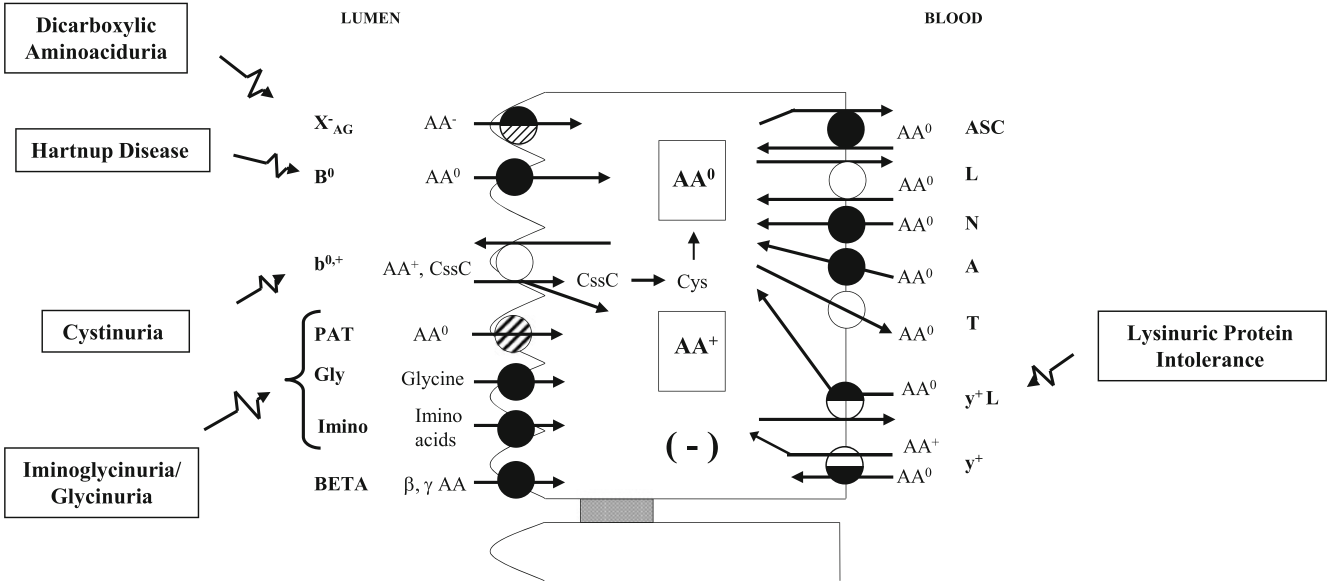

LUMEN
BLOOD
Dicarboxylic Aminoaciduria
Hartnup Disease
Cystinuria
Iminoglycinuria/ Glycinuria
Lysinuric Protein Intolerance
X-AG
B0
b0,+
PAT
Gly
Imino
BETA
AA-
AA0
AA+, CssC
AA0
Glycine
Imino acids
β, γ AA
CssC
Cys
AA0
AA+
(-)
AA0
AA0
AA0
AA0
AA0
AA0
AA+
AA0
ASC
L
N
A
T
y+L
y+

Fig. 1 Summary of amino acid transport mechanisms in the proximal tubule. Filled circles indicate Na^+-dependent, active, carrier-mediated cotransport or antiport (exchange). Dashed circles indicate H^+-dependent, active, carrier-mediated cotransport. Empty circles indicate Na^+-independent antiport or uniport (facilitated diffusion). Half-filled, half-dashed, or half-empty circles indicate various combinations of the above. Known or suspected transport defects leading to different hereditary aminoacidurias are depicted; AA^0, neutral amino acids; AA^+, basic amino acids; AA^-, acidic amino acids; CssC, cystine; Cys, cysteine. See text for details

heterogeneity of Na^+-linked amino acid uptake systems with respect to kinetic characteristics has been demonstrated for several amino acids in BBMV derived from pars convoluta and pars recta of the proximal tubule [1]. The recognition of several transport systems for the same amino acid as well as their axial heterogeneity is of major importance in understanding the pathophysiology of hereditary aminoaciduria.

One or more Na^+ ions are transported for each amino acid molecule translocated, and with most amino acids, this process is electrogenic-positive driven by a negative cell interior [1, 12]. Na^+-amino acid stoichiometry determines the electrogenicity and efficiency of the transport system [7]. Additional ions besides Na^+ are involved in the translocation of the amino acid carrier complex across the brush-border membrane [1]. Taurine [13], glycine [14], and proline [15] transport, for example, operates by means of 2 or 3 Na^+/1 Cl^-/1 amino acid carrier complex.

Specificity and Classification of Amino Acid Transport Systems

It has been well established that several distinct chemical group-specific Na^+-dependent transport systems for amino acids exist in the tubular luminal membrane [1–3]. Evidence for these systems has been derived from a variety of microperfusion experiments and vesicle studies and, in humans, from the existence of inborn errors of renal tubular transport that can be explained only by defects in specific transport pathways [1, 2, 16]. These include systems for dibasic (cationic) amino acids, cystine, acidic (anionic) amino acids, neutral α-amino acids, imino acids, glycine, and β- and γ-amino acids. In addition to separate systems for L-cystine and the dibasic amino acids (L-lysine, L-arginine, and L-ornithine), these amino acids likely share a common transport pathway [1, 2], as suggested by the urinary hyperexcretion of all four amino acids in cystinuria (see Cystinuria below).

The broad-specificity transport pathway for neutral α-amino acids, which is a low-affinity, high-capacity system, is located in the proximal convoluted tubule [16]. However, there are several alternative specific renal transport systems for neutral amino acids, including high-affinity systems located in the proximal straight tubule. The presence of such pathways is also suggested by the finding of isolated transport defects for neutral amino acids (see Neutral Aminoaciduria). The imino acids proline and hydroxyproline are reabsorbed by at least three systems: a low-affinity/high-capacity system in the proximal convoluted tubule shared with glycine; an imino acid-specific, high-affinity/low-capacity system in the proximal straight tubule; and a separate high-affinity/low-capacity system for glycine in the late proximal tubule [11].

Classification of Amino Acid Transport Systems by Chemical Properties/Substrate Specificity

The investigation of amino acid transport pathways in the plasma membrane of mammalian cells has delineated several transport systems which are classified according to their chemical properties/substrate specificity [2, 6, 17, 18, 19] (Table 1; Fig. 1). Most of these transport systems also have been identified in the kidney [2, 6, 17, 18, 20]. The neutral amino acid transport systems include the Na^+-dependent, concentrative B^0 system (for most neutral amino acids), A and ASC systems (for small neutral amino acids), and N system (for glutamine, asparagine, and histidine); the Na^+- and Cl^- -dependent Imino system (for proline and hydroxyproline), Gly system (for glycine and alanine), and BETA system (for taurine, β-alanine, and γ-aminobutyric acid); the H^+ gradient-dependent PAT system (for imino acids and glycine); as well as the Na^+-independent, nonconcentrative L system (for bulky neutral, branched-chain amino acids and cysteine) and the T system (for aromatic amino acids). The cationic amino acid transport systems include the $b^{0,+}$ system (for dibasic and neutral amino acids including cystine), y^+ system (for the dibasic amino acids lysine, arginine, and ornithine), and y^+L system (for dibasic and neutral amino acids excluding cystine). The anionic amino acid transport system includes the Na^+- and H^+- dependent X^-_{AG} system (for acidic amino acids). Systems B^0,

Table 1 Amino acid transport systems operating in the renal tubule

Amino acid transport system	Cloned cDNA	Gene	Amino acids transported	Mechanism of action	Localization in proximal tubule	Cellular localization
I. Neutral						
B^0	B^0AT1	*SLC6A19*	Most neutral	Na^+-AA cotransport	EPT	BBM
Gly	XT2 (B^0AT3)	*SLC6A18*	Glycine, alanine	2–3 $Na^+/1Cl^-/$ 1AA cotransport	LPT	BBM
Imino	IMINO (SIT1)	*SLC6A20*	Proline, hydroxyproline	2–3 $Na^+/1Cl^-/$ 1AA cotransport	LPT	BBM
BETA	TAUT BGT-1	*SLC6A6* *SLC6A12*	Taurine, β-alanine Betaine, GABA	2–3 $Na^+/1Cl^-/$ 1AA cotransport	LPT	BBM
PAT	PAT2	*SLC36A2*	Proline, glycine, alanine	H^+-AA cotransport	EPT	BBM
L	4F2hc/ LAT-1 4F2hc/ LAT-2	*SLC3A2/ SLC7A5* *SLC3A2/ SLC7A8*	Large, branched-chain neutral	AA antiport	EPT	BLM
T	TAT1	*SLC16A10*	Aromatic	AA uniport	EPT	BLM
A	SNAT2,4	*SLC38A2,4*	Short-chain neutral	Na^+-AA cotransport	EPT and LPT	BLM
N	SNAT 3,5	*SLC38A3,5*	Glutamine, asparagine, histidine	1 Na^+/AA cotranport 1 H^+ antiport	LPT	BLM
ASC	ASCT2 (EAAT)	*SLC1A5*	Short-chain neutral	Na^+-dependent AA antiport	EPT and LPT	BLM
II. Cationic						
$b^{0,+}$	rBAT/ $b^{0,+}$AT	*SLC3A1/ SLC7A9*	Cationic and neutral (including cystine)	AA antiport	EPT and LPT	BBM
y^+L	4F2hc/ y^+LAT-1 4F2hc/ y^+LAT-2	*SLC3A2/ SLC7A7* *SLC3A2/ SLC7A6*	Cationic and neutral (excluding cystine)	Na^+-dependent AA antiport	EPT	BLM
y^+	CAT 1	*SLC7A1*	Cationic and neutral (excluding cystine)	AA uniport (facilitative transport)	EPT and LPT	BLM
III. Anionic						
X^-_{AG}	EAAT3 (EAAC1)	*SLC1A1*	Anionic	3 $Na^+/1\ H^+/$ 1AA cotransport 1 K^+-antiport	LPT	BBM

EPT early proximal tubule (S1, S2 segments), *BBM* brush-border membrane
LPT late proximal tubule (S3 segment), *BLM* basolateral membrane

X^-_{AG}, Imino, Gly, β, PAT, and $b^{0,+}$ operate in the luminal membrane, whereas systems A, ASC, N, L, T, y^+, and y^+L operate in the basolateral membrane [2, 18, 20] (Fig. 1). The basolateral membrane-bound systems ASC, L, y^+, and y^+L as well as the luminal membrane-bound $b^{0,+}$ system function as antiport (exchange) systems [3, 18, 19] (Fig. 1).

Classification of Amino Acid Transport Systems by Sequence Homology/ Structural Features

Over the past two decades, molecular biology techniques had led to elucidating the molecular structure of various membrane-bound transport proteins, including amino acid transporters. The identification of brush-border membrane transporters was pioneered by Hediger et al. [45], who cloned the small intestinal Na^+-glucose cotransporter (see Molecular Biology of Na^+-Glucose Cotransporters) using expression cloning in *Xenopus* oocytes. Similar approaches have been used to clone, functionally express, and sequence various amino acid transporters. This has led to the classification of amino acid transporters based on gene homology using the solute carrier family (SLC) nomenclature introduced by the Human Genome Organization (HUGO) [22].

The sequence homology and structural features of amino acid transporters have led to their categorization into seven different gene families [2, 3, 18, 23, 24, 25] (Table 1): **(i)** the SLC1 or EAAT/ ASCT family (Na^+-dependent anionic/neutral amino acid transporters), **(ii)** the SLC6 family [Na^+- (and $C1^-$ -)dependent transporters], **(iii)** the SLC3/SLC7 or HAT family (cationic/neutral amino acid transporters), **(iv)** the SLC7 or CAT/LSHAT family (cationic/neutral amino acid transporters), **(v)** the SLC16 or TAT family (aromatic amino acid transporters), **(vi)** the SLC36 or PAT family (proton-coupled imino acid transporters), and **(vii)** the SLC38 or SNAT family (Na^+-coupled neutral amino acid transporters). As opposed to all other SLC families that contain multiple transmembrane domains [25], members of the SLC3 family function as components or subunits of heteromeric carriers (see Cystinuria).

Hereditary Aminoacidurias

Aminoacidurias are a group of disorders in which a single amino acid or a group of amino acids are excreted in excess amounts in the urine due to decreased tubular reabsorption. The defective tubular reabsorption is assumed to result from a genetic defect in a specific transport system that directs the reabsorption of these amino acids under normal conditions. Some of these disorders also involve a similar transport abnormality in the intestine. As opposed to inborn errors of amino acid metabolism, in which plasma levels of amino acids are elevated, resulting in overflow aminoaciduria, plasma levels of amino acids in hereditary aminoacidurias are largely normal. The aminoacidurias are generally categorized into five major groups according to the group-specific transport pathway presumed to be affected (Table 2). The groups are further subdivided into several disorders, based on the profile of the affected amino acids within that group (Table 2; Fig. 1).

Cationic Aminoaciduria

Four distinct cationic amino acid transport defects have been identified: *(a)* cystinuria, *(b)* hyperdibasic aminoaciduria, *(c)* lysinuric protein intolerance, and *(d)* isolated lysinuria. These diseases differ in identified or putative transport systems affected, pathophysiology, organs involved, and clinical features. Cystinuria is the prototype for this group of hereditary aminoacidurias.

Cystinuria Cystinuria (OMIM 220100) is an autosomal-recessive disorder of amino acid transport characterized by excessive urinary excretion of cystine and the dibasic amino acids lysine, arginine, and ornithine that have structural similarity (two amino groups separated by four to six chemical bonds). The pathogenic mechanism of cystinuria is defective transepithelial transport of these amino acids in the proximal tubule and the small intestine [26]. The very low solubility of cystine in the urine results in cystine stone formation in homozygous patients. Lysine, arginine, and ornithine do not form urinary stones as they are much more soluble than cystine. Urinary cystine calculi may produce considerable morbidity including urinary obstruction, colic, infection, and, in severe cases, loss of kidney function. Cystinuria accounts for 1–2% of all cases of urolithiasis and 4–5% of urolithiasis cases in children [27, 28]. The defective gastrointestinal transport

Table 2 Hereditary aminoacidurias

Disorder	**Defective gene**	**Locus**	**Amino acid transport system defective**	**Individual amino acids affected**	**Localization of defect**	**Mode of inheritance**	**Prevalence**	**Clinical manifestations**	**Organs involved**	**OMIM no.***
I. Cationic aminoaciduria										
1) Cystinuria Type A Type B	*SLC3A1* *SLC7A9*	2p16.3 19q13.1	rBAT (heavy subunit of $b^{0,+}$) $b^{0,+}$AT (light subunit of $b^{0,+}$)	Cystine, lysine, arginine, ornithine	BBM BBM	AR AR (incomplete penetrance)	1:7000–1:15000	Urolithiasis	Kidney, intestine	220100 600918
2) Hyperdibasic aminoaciduria Type I			Dibasic amino acids	Lysine, arginine, ornithine	BBM	AD	2 families reported	Mental retardation	Kidney, intestine	222690
3) Lysinuric protein intolerance (hyperdibasic aminoaciduria Type II)	*SLC7A7*	14q11	y^{+}LAT-1 (light subunit of y^{+}L)	Lysine, arginine, ornithine	BLM	AR	1:60,000 (Finland)	Protein malnutrition, failure to thrive, hyperammonemia, seizures, coma	Kidney, intestine, lungs, bones	222700
4) Isolated lysinuria			Lysine (?)	Lysine	BBM (?)	AR	1 patient reported	Failure to thrive, seizures, mental retardation	Kidney, intestine	
II. Neutral aminoaciduria										
1) Hartnup disease	*SLC6A19*	5p15.33	B^{0}(neutral, monoamino monocarboxylic α-amino acids)	Alanine, serine, threonine, valine leucine, isoleucine, phenylalanine tyrosine, tryptophan, histidine, glutamine, asparagine	BBM	AR	1:20,000	Skin rash, cerebellar ataxia, psychiatric illnesses	Kidney, intestine	234500

2) Methioninuria			Methionine	Methionine	BBM	AR	2 cases reported	Edema, seizures, mental retardation	Kidney, intestine	250900
3) Histidinuria			Histidine	Histidine	BBM	AR	4 cases reported	Mental retardation	Kidney, intestine	235830
III. Imminoaciduria and glycinuria										
1) Imino-glycinuria	*SLC36A2* (PAT2) *SLC6A20* (IMINO)	5q33.1 3p21.3	Imino acids and glycine	Proline, hydroxy-proline, glycine	BBM	AR	1:15,000	Benign	Kidney, intestine	242600
2) Isolated glycinuria	*SLC6A18* (XT2) (?)	5p15.33	Glycine	Glycine	BBM	AD	2 families reported	Benign	Kidney	138500
IV. Dicarboxylic aminoaciduria	*SLC1A1* (EAAT3)	9q24	X^-_{AG} (acidic amino acids)	Glutamate, aspartate	BBM	AR	1:29,000 (French-Canadian)	Benign	Kidney, intestine	222730
V. β-amino-aciduria (mouse)			β-amino acids	Taurine	BLM (?)	AR		Benign	Kidney	

BBM – brush border membrane; *BLM* – basolateral membrane; *AR* – autosomal recessive; *AD* – autosomal dominant

* Online Mendelian Inheritance in Man (database at http://www.ncbi.nlm.nih.gov/omim)

of cystine and dibasic amino acids in cystinuria does not result in intestinal disease.

Transport Defect Normally only 1% of the filtered cystine and dibasic amino acids is excreted. In cystinuria, cystine clearance may be near or equal to the glomerular filtration rate (GFR). Lysine and ornithine clearance is 30–80% of the GFR, whereas arginine excretion is lower [28]. Studies using isolated cortical tubules and BBMV [1, 29] have provided evidence for three brush-border membrane-bound and two basolateral membrane-bound carrier systems for cationic amino acids in the kidney. Transport at the brush-border membrane occurs by a system shared by cystine and the dibasic amino acids, a system specific for cystine, and a system specific for dibasic amino acids. It is the shared high-affinity, low-K_m system located in the S3 segment of the proximal tubule and identified as system $b^{0,+}$ (see Specificity and Classification of Amino Acid Transport Systems; Figs. 1 and 2) that is defective in cystinuria. The low-affinity, high-K_m, cystine-specific system and the low-affinity, dibasic amino acid-specific system, both located in the S1–S2 segments of the proximal tubule, have remained unidentified at the cellular/molecular level. The antiluminal membrane harbors two specific systems, one for dibasic amino acids identified as system y^+L (see Specificity and Classification of Amino Acid Transport Systems; Figs. 1 and 2) and one for cystine, but no shared system. These luminal and basolateral transport mechanisms mediate uptake and efflux of these amino acids across the basolateral membrane, respectively. The brush-border membrane of the intestinal cell has a single high-affinity, low-K_m, shared $b^{0,+}$ transport system for cystine and dibasic amino acids that is defective in cystinuria. Like kidney cells, intestinal cells have two basolateral membrane-bound, unshared/specific transport systems, one (system y^+L) for dibasic amino acids and one for cystine [2, 3]. The basolateral membrane-bound y^+L transporter for dibasic amino acids in the kidney and the intestine is defective in lysinuric protein intolerance (see Lysinuric Protein Intolerance). Amino acid transport in parenchymal cells and leukocytes from cystinuric patients is not impaired [2, 3] because

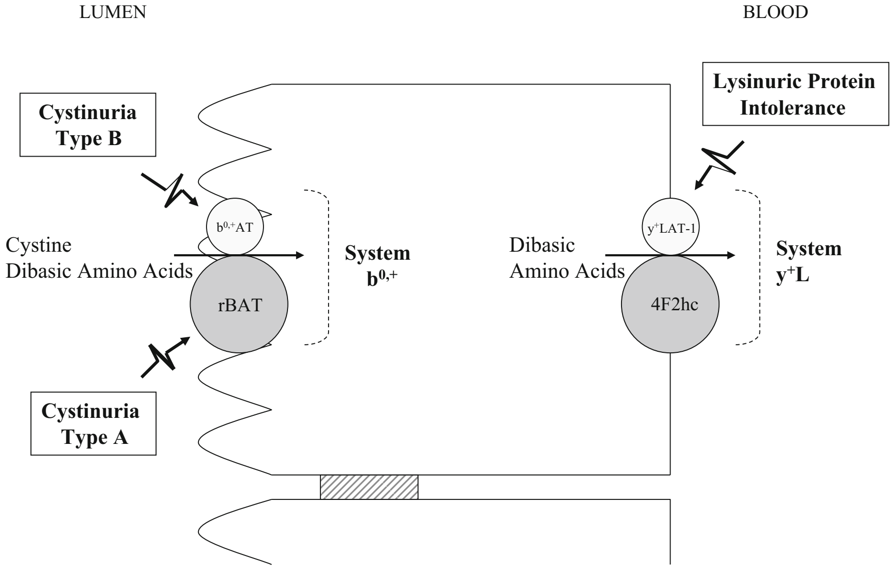


Fig. 2 Transport pathways for cystine and dibasic amino acids at the luminal and basolateral membranes of a proximal tubular cell. *Large circles* represent the heavy subunits and *small circles* the light subunits of the heteromeric amino acid transporters $b^{0,+}$ and y^+L. Hereditary aminoacidurias caused by defects in these transporters are depicted. (Modified with permission from ref. #45)

the transport defects are not found in the plasma membrane of these cells.

Prevalence Cystinuria is inherited in an autosomal-recessive fashion. Among the various types of aminoacidurias, it is a common disorder with an overall prevalence of 1:7000 to 1:15,000 and estimated gene frequency of 0.01 [26]. A very high prevalence of 1:2500 is observed in Israeli Jews of Libyan origin [30].

Classification Three phenotypes of cystinuria (types I, II, and III) have traditionally been described on the basis of the urinary excretion of cystine in the obligate heterozygous parents [31, 32]. More recently, a genotypic classification of cystinuria was introduced [33]. Most patients have genotype AA or BB, which denotes two mutated alleles in the genes *SLC3A1* or *SLC7A9*, respectively (see Molecular Genetics). A0 and B0 denote the identification of only one mutated allele, i.e., heterozygous carriers. Carriers with the A0 genotype usually have normal cystine excretion, while carriers with the B0 genotype have often elevated cystine excretion; they very rarely develop stones [34].

Molecular Genetics The heteromeric amino acid transporters (HAT), implicated in cystinuria (and in lysinuric protein intolerance; see later), are composed of a heavy subunit (SLC3; HSHAT) and a light subunit (SLC7; LSHAT) which are linked by a conserved disulfide bridge [23, 35, 36, 37, 38] (Fig. 3). The heavy subunit appears essential for trafficking the transporter to the membrane, whereas the light subunit confers the transporter function [25, 37].

Using linkage analysis in 17 cystinuric families, Pras et al. demonstrated linkage between cystinuria and three genetic markers on chromosome 2p, providing strong evidence that rBAT/SLC3A1 was the gene causing the disease [39]. Calonge et al. identified six specific mutations in the *SLC3A1* gene that segregated with a cystinuria phenotype, thereby establishing the rBAT gene, which encodes the heavy subunit of $b^{0,+}$, as a cystinuria gene (Fig. 2) [40]. To date, a total of 163 different rBAT mutations have been reported in patients with type A cystinuria [26, 41]. These mutations include nonsense, missense, splice site, and frameshift mutations, as well as large deletions and chromosome

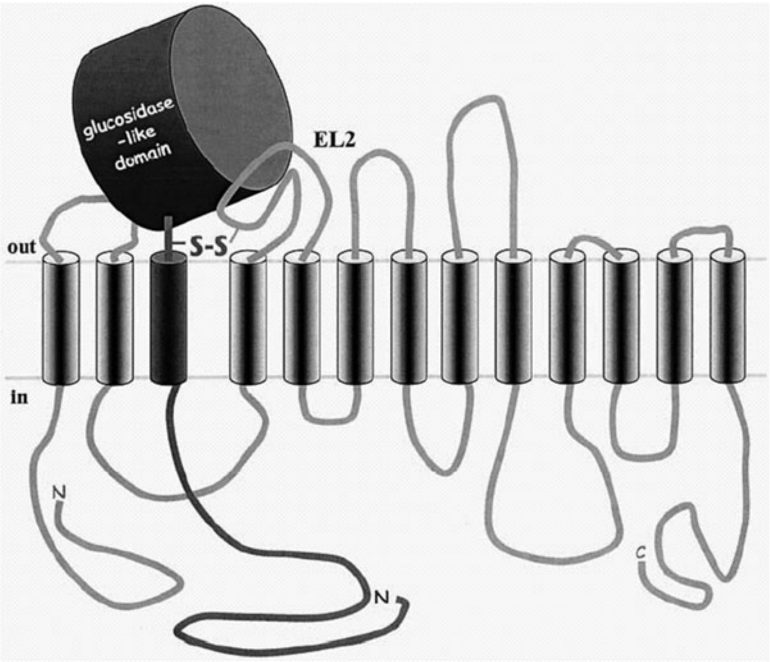


Fig. 3 Schematic representation of the heteromeric amino acid transporters (HAT). The heavy subunit of HAT (HSHAT; dark gray) is linked by a disulfide bridge to the corresponding light subunit of HAT (LSHAT; light gray). The cysteine residues involved in this bond (S-S) are located in an extracellular domain, following the transmembrane (TM) domain of HSHAT, and in the proposed extracellular loop 2 (EL2) of LSHAT. Loops and TM domains are simplified and not drawn to scale. See text for details. (Adapted with permission from ref. #38)

rearrangements [26, 34, 42]. Defective transport of cystine and dibasic amino acids has been demonstrated for many of these mutations when expressed in *Xenopus* oocytes [43]. The most commonly occurring mutation is methionine 467 to threonine (M467T), which causes a defect in trafficking to the plasma membrane [44, 45]. This trafficking defect, which has also been demonstrated for other rBAT mutations [46], is consistent with the proposed role of rBAT as a chaperon for the corresponding light subunit in the heteromeric amino acid transporter.

In 1997, Wartenfeld et al. [47] and Bisceglia et al. [48] demonstrated that cystinuria in other patients were linked to a locus on chromosome 19q13.1 (type B cystinuria). Subsequently, the International Cystinuria Consortium (ICC) [49] identified the gene *SLC7A9* which encodes the 487 amino acid protein $b^{0,+}$AT that belongs to the family of light subunits of amino acid transporters (LSHAT; Table 1). *SLC7A9* mutations were found in Spanish, Italian, North American, and Libyan-Jewish cystinuria patients [49, 50]. The G105R mutation is the most frequent *SLC7A9* mutation in the ICC cohort of patients [36]. The mutation in Jews of Libyan origin is valine 170 to methionine (V170M), which leads to complete loss of $b^{0,+}$AT amino acid uptake activity when cotransfected with rBAT in COS cells. To date, 118 different $b^{0,+}$AT mutations have been identified [26, 41].

Large-scale rearrangements represent between 17% and 21% of all found variants [42, 54]. The unexplained cases might be due to mutations outside the open reading frame of the *SLC7A9* and *SLC3A1* genes (intronic or promoter regions) or due to mutations in unidentified genes [26]. There may be mixed genotype groups such as AB, where individuals have one pathogenic variant in *SLC3A1* and one in *SLC7A9*, and AAB and BBA if there are more than two pathogenic variants pertaining to different genes. The ICC has reported that type A, type B, and type AB account for 38%, 47%, and 14% of cystinuria patients in their registry [34, 35].

Jaeken et al. described an autosomal-recessive disorder, hypotonia-cystinuria syndrome (MIM 606407), characterized by generalized hypotonia at birth, failure to thrive, growth retardation, minor facial dysmorphism, mild to moderate intellectual disability, and cystinuria type A [51]. The syndrome is caused by microdeletions of *SLC3A1* and a prolyl oligopeptidase-like gene (*PREPL*) which are adjacent on chromosome 2p21 [51]. Several similar disorders have been described in which type A cystinuria is associated with hypotonia-related syndromes due to the combined deletion of *SLC3A1* and contiguous genes [52].

Diagnosis and Clinical Features The diagnosis of cystinuria can be made by analysis of kidney stones, observation of cystine crystals in the urinary sediment, or detection of an abnormal excretion of cystine and dibasic amino acids in the urine in adults and children [53]. The simplest diagnostic test is the microscopic examination of the urinary sediment of a freshly voided morning urine [27]. The presence of typical flat hexagonal cystine crystals is diagnostic. The cyanide-nitroprusside test (CNT) is a rapid qualitative test that has been proposed as screening method for cystinuria. However, due to its low reproducibility and sensitivity and the use of hazardous and unstable reagents, most consider this test to be outdated [27]. The definite test is a measurement of urine cystine and dibasic amino acid concentration by ion exchange chromatography. The upper limits of normal are 18, 130, 16, and 22 mg/g creatinine for cystine, lysine, arginine, and ornithine, respectively [28]. While assessing for the possibility of cystinuria, it is important to exclude other conditions associated with increased urinary cystine excretion, including tubular immaturity in young infants, generalized aminoaciduria (Fanconi syndrome), and organic acidemias [27, 28].

Cystinuria usually presents with renal colic. In most patients, the first stone is detected during childhood or adolescence. Infection, hypertension, or renal failure may also develop [27]. Cystinuria occurs with equal frequency in males and females; one study reported that males were more severely affected [33], although other case series did not confirm that finding [54, 55]. Most patients have recurrent stone formation; however, not all patients with *SLC3A1* and *SLC7A9* mutations develop urinary calculi throughout their lives, and, in stone formers, age of onset of the lithiasis can vary substantially [26].

Cystine stones are radiopaque because of the density of the sulfur molecule and on a roentgenogram appear smooth. Occasionally, they form staghorn calculi. Cystine also may act as a nidus for calcium oxalate so that mixed stones may be found. Factors contributing to mixed stone formation in cystinuria include alkalinization of urine and urinary tract infections. Cystinuric patients who receive a kidney transplant have normal urinary cystine and dibasic amino acid excretion following transplantation [56].

Treatment The mechanisms involved in cystine kidney stone formation are related to the high concentration of cystine and its low solubility at normal urinary pH. Cystine crystalluria occurs when the cystine content of the urine exceeds 300 mg/L at pH 4.5–7. Cystine solubility increases sharply at a urine pH above 7 [27]. The major therapeutic approaches to cystinuria are designed to increase the solubility of cystine, reduce excretion of cystine, and convert cystine to more soluble compounds [26, 27]. Therapies used in the management of cystinuria include the following:

- Increased oral fluid intake to increase urine volume and cystine solubility. Because cystinuric patients excrete 0.5–1 g cystine/day, intake of 3–4 L is required to keep the urinary cystine concentration below 250 mg/L [53]. In children it is recommended to promote enough fluid intake to have a 24-h urine volume of more than 2 liters/1.73 m^2 [53]. Patients should develop a 24-h schedule for drinking and voiding, with particular attention to nighttime hours, when urine may become supersaturated with cystine. Rigid adherence to fluid therapy is effective in approximately 70% of patients [27].
- Oral alkali can further increase cystine solubility in the urine when combined with augmented fluid intake. A urine pH of 7.5–8 can be achieved by oral bicarbonate or citrate in divided daily doses. Because high sodium intake increases cystine excretion (see below), potassium citrate (60–80 mEq/day) is preferred [28]. Because urine alkalinization may result in formation of mixed calcium-containing stones, adherence to high fluid intake is crucial.
- Dietary therapy can reduce cystine production and excretion. Reduced methionine intake lowers cystine production, although intake should not be lower than the physiological requirement (i.e., 1200–1400 mg/day in adults) [54]. Protein restriction is not advisable in children as that may affect their growth. Urinary excretion of cystine and dibasic amino acids in cystinuric patients correlates with urinary sodium excretion [57]. Hence, dietary sodium restriction is also recommended for the treatment of cystinuria [26, 58].
- Pharmacologic therapy can increase cystine solubility and decrease cystine excretion. The sulfhydryl-binding compound D-penicillamine (β-dimethylcysteine) leads to the formation of the mixed disulfide penicillamine-cysteine following a disulfide exchange reaction. This mixed disulfide is far more water-soluble than cystine. Penicillamine, given at a maximum dosage of 1.2 g/24 h (20–30 mg/kg in children), is highly effective in reducing urinary cystine excretion. Unfortunately, penicillamine has serious side effects including rash, fever, arthralgia, nephrotic syndrome, pancytopenia, and loss of taste. Penicillamine also impairs pyridoxine metabolism and increases copper and zinc excretion in the urine. Mercaptopropionyl glycine (tiopronin; Thiola), another agent undergoing a disulfide exchange reaction, is as effective as D-penicillamine in the treatment of cystinuria. While some have suggested that serious adverse events are less common with tiopronin, a large recently published study showed that the incidence of adverse effects was similar for the former and penicillamine [55, 59]. Hence, cystine-binding drugs should be reserved for patients unresponsive to conservative management, and stepwise dosing is recommended [27].

Studies examining the efficacy of the angiotensin-converting enzyme inhibitor, captopril, and meso-1,3-dimercaptosuccinic acid

(DMSA) (both sulfhydryl compounds able to build highly soluble disulfides with cysteine) in cystinuria have yielded conflicting results [60, 61]. Hence, these agents are not recommended as therapeutic modalities in cystinuria. Finally, as recently suggested by Goodyer's group [41], cystinuria, like several other hereditary kidney diseases, could be amenable to therapy by a new class of aminoglycosides with increased translational readthrough of nonsense mutations and reduced toxicity. Cystinuria type A patients with homozygous nonsense mutations in *SLC3A1* are most likely to benefit from this potential therapy since even partial restoration of gene function might reduce urinary cystine to levels below the solubility threshold [41].

- Several urologic procedures have been used to treat cystine stones. Extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), or percutaneous nephrolithotomy (PCNL) are all options for treating cystine stones. Although cystine stones are often considered relatively ESWL-resistant, ESWL may be offered to cystinuria patients, including children, with previous treatment success or to avoid invasive procedures. When PCNL is considered, miniaturized techniques may offer equivalent stone clearance rates to that with standard PCNL but with reduced renal insult [53].

In summary, the mainstays for therapy in cystinuria include hydration, alkalinization of the urine, and dietary sodium restriction. Full compliance with this regimen results in significantly reduced urinary cystine excretion and good long-term prognosis in most patients. Pharmacologic treatment with sulfhydryl agents should be reserved for patients in whom conservative therapy fails. The use of increased translational readthrough in the treatment of cystinuria remains to be established. Urologic intervention is indicated in selected patients with severe obstructing stones.

Lysinuric Protein Intolerance (LPI) (Hyperdibasic Aminoaciduria Type II) Lysinuric protein intolerance is a rare autosomal-recessive disorder characterized by excessive urinary excretion of dibasic amino acids (especially lysine), normal cystine excretion, and poor intestinal absorption of dibasic amino acids [62, 63]. Plasma values of dibasic amino acids are low. The disease is most common in Finland, where the prevalence of the disease is 1:60,000 [62]. About 140 patients, Finish as well as non-Finish, have been described [62, 63, 64]. Homozygous patients have massive dibasic aminoaciduria as well as hyperammonemia after a protein load; heterozygotes have normal urinary amino acid excretion but impaired renal and intestinal transport of dibasic amino acids with increased protein loads. The clinical manifestations in subjects homozygotes for LPI often mimic urea cycle disorders, including having digestive and neurological signs and symptoms (hypotonia, lethargy, ataxia, seizure, and hyperammonemic coma after high protein intake). Malnutrition is often observed and is responsible for growth delay. Most patients also have cytopenia, hepatosplenomegaly, high ferritinemia, and hypertriglyceridemia attributed to chronic hemophagocytic lymphohistiocytosis [64, 65]. Pulmonary alveolar proteinosis is observed in up to 70% of the patients [66]. Osteopenia and severe osteoporosis sometimes leading to pathological fractures can occur.

The pathogenic mechanism of LPI appears to be a defective transport of dibasic amino acids in the basolateral membrane of renal and intestinal epithelial cells, resulting in impaired efflux from the cell to the interstitium [67, 68]. This has been confirmed by a measurement of fluxes in jejunal biopsy specimens from LPI patients [67] as well as by the observation that infusion of citrulline to these patients results in massive argininuria and ornithinuria [68]. Citrulline is reabsorbed from the tubular lumen by a neutral amino acid transport mechanism and is converted to arginine and ornithine in the renal cell. Impaired exit at the basolateral membrane results in backflux of accumulated arginine and ornithine across the brush-border membrane. The high-affinity, specific dibasic amino acid transporter y^+L is the protein affected in this disease (69, 70; Fig. 1). Because basolateral membrane transporters of epithelial cells and plasma membrane transporters of parenchymal cells are homologous carriers, it is not surprising that granulocytes [71] and cultured skin fibroblasts [72] from patients with LPI show impaired transport of dibasic amino acids. Erythrocytes from LPI patients, which do not have the y^+L system, have normal cationic amino acid transport.

It is presumed that the poor intestinal absorption and excessive renal loss of dibasic amino acids deprive hepatic cells of ornithine and arginine, which are necessary for urea production [62, 70]. This results in protein intolerance, hyperammonemia, and limited urea formation. These presumptions are supported by the observation that L-citrulline supplements improve protein tolerance in LPI patients [68]. L-citrulline, a metabolic precursor of ornithine and arginine, is absorbed in the intestine, enters the hepatic cell via neutral amino acid transport mechanisms, is metabolized in the liver to ornithine and arginine, and restores the pathway for ammonia disposal [62]. Several abnormalities seen in LPI patients including glomerular dysfunction [73], erythroblastophagia, and alveolar proteinosis [74] suggest that the immune system is deranged in some of these patients.

The molecular genetics of LPI has been elucidated. Linkage studies in Finnish and non-Finnish LPI families localized the gene associated with LPI to 14q11–13 [75, 76]; subsequently, Torrents et al. identified mutations in the *SLC7A7* gene as causative for LP1 [77]. Normally, y^+LAT-1 (*SLC7A7*), a member of the family of light subunits, combines with 4F2hc (*SLC3A2*), a member of the heavy subunit family, to form a heteromeric amino acid transporter (HAT) [35, 78] (Figs. 2 and 3). The 4F2hc/y^+LAT-1 transporter comprises the amino acid transport system y^+L that causes efflux of basic amino acids at the basolateral membrane of epithelial cells [45] (see Specificity and Classification of Amino Acid Transport Systems; Figs. 1 and 2). The heavy subunit, 4F2hc (*SLC3A2*), is essential for trafficking the transporter to the membrane, whereas the light subunit, y^+LAT-1 (*SLC7A7*), confers the transporter function [25, 37]. In 1999, two groups demonstrated that mutations in *SLC7A7* cause LPI in Finnish, Italian, and Spanish patients [79, 80]. To date, approximately 50 *SLC7A7* mutations, found along the entire *SLC7A7* gene, have been identified in LPI patients from different ethnic groups [70, 81]. Expression studies in *Xenopus* oocytes have shown that several *SLC7A7* LPI mutations result in y^+LAT-1 proteins that fail to dimerize with 4F2hc and reach the plasma membrane [82]. While 4F2hc (*SLC3A2*) is ubiquitously expressed, y^+LAT1 (*SLC7A7*) is primarily expressed in tissues affected in LPI including the kidney, small intestine, lung, and white blood cells [35, 77].

No LPI mutations have been identified in the *SLC3A2* gene, which suggests that these may be lethal [25]. Indeed, 4F2hc is responsible for the trafficking of at least six amino acid transporter light subunits [36], including y^+LAT-1, and mediates integrin signaling [83]. Finally, 4F2hc knockout in mice is lethal [84].

LPI has poor genotype/phenotype correlations. Finnish patients, who share the same founder mutation, have variable clinical pictures [70, 82]. Similarly, both interfamilial and intrafamilial phenotypic variability have been observed in Italian LPI patients carrying the same mutation [63]. This suggests that, in addition to *SLC7A7* mutations, currently unknown genetic modifiers have a role in the pathogenesis of LPI [69].

Therapy for LPI includes protein restriction to prevent hyperammonemia. In addition, oral citrulline supplements help correct the hepatic deficiency in ornithine and arginine, resulting in clinical improvement and catch-up growth [64].

Hyperdibasic Aminoaciduria Type I Hyperdibasic aminoaciduria type I (HDBA I) is an autosomal-dominant cationic aminoaciduria that has been described in two families with several heterozygous and a single homozygous patient [85, 86]. This disease is characterized by impaired renal reabsorption and intestinal absorption of the dibasic amino acids lysine, arginine, and ornithine, but not of cystine. Plasma values of dibasic amino acids are normal and patients have no protein intolerance or hyperammonemia. The single homozygous patient had mental retardation [86]. HDBA I heterozygotes have modest cationic aminoaciduria, whereas LPI heterozygotes have no hyperaminoaciduria. Some speculate that the brush-border, membrane-bound, high-capacity transporter for dibasic amino acids, which excludes cystine, is defective in this disease. The molecular identity of the transporter affected by this disease is unknown.

Isolated Lysinuria This disease was first reported by Omura et al. who described a single child with increased urinary excretion and impaired intestinal absorption of lysine, low plasma lysine levels, and normal renal and intestinal transport of ornithine, arginine, and cystine [87]. The patient did not have hyperammonemia but had poor overall growth and mental retardation, likely secondary to the deficient lysine. This case implies the existence of a selective transport system for lysine in the kidney and the intestine. Such a system has not yet been identified in physiologic or molecular studies.

Neutral Aminoaciduria

Three distinct disorders of neutral amino acid transport have been identified: Hartnup disorder, methioninuria, and histidinuria.

Hartnup Disease Hartnup disease, which may have afflicted Julius Caesar and his family [88], was first recognized in two siblings in England in 1956. This disease is characterized by intestinal malabsorption and massive urinary levels of the neutral monoamino monocarboxylic amino acids: alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine, glutamine, and asparagine [89, 90]. Most patients also have increased excretion of indolic compounds that originate in the gut from bacterial degradation of tryptophan [91]. Transport of other neutral amino acids including cystine, imino acids, glycine, and β-amino acids is unaffected. The disease has autosomal-recessive inheritance and has an estimated incidence of 1:20,000 live births. Heterozygous carriers have normal urinary acid excretion under physiologic conditions. Clinical features in homozygous patients may include photosensitive rash, cerebellar ataxia, and a variety of psychiatric manifestations mimicking pellagra [89, 90]. The pellagra-like manifestations are primarily caused by intestinal malabsorption and urinary loss of tryptophan, an amino acid that is required for niacin synthesis. The diagnosis should be suspected in any patient with pellagra who has no history of niacin or nicotinamide deficiency and should be made by chromatographic analysis of the urine [89].

A defect in the broad-specificity neutral, α-amino acid transport mechanism in the renal and intestinal brush-border membrane is presumed to be the pathogenic mechanism underlying this disorder [16]. Several lines of evidence support the contention that the transport defect is localized to the brush-border membrane [16]. First, plasma amino acid response in patients with Hartnup disease was somewhat restored after oral feeding of free α-amino acids but not when α-amino acid dipeptides were given orally [92]. Dipeptides are presumed to be reabsorbed by a dipeptide-specific brush-border membrane transporter and hydrolyzed within the intestinal cell; the free amino acids can then exit the cell via an apparently nondefective basolateral membrane-bound carrier in these patients. Secondly, tryptophan transport is normal in various parenchymal cells from patients with Hartnup disease including leukocytes, placenta, and cultured skin fibroblasts [89]. As indicated earlier, amino acid transporters in plasma membranes of parenchymal cells are homologous to basolateral membrane transporters in epithelial cells, making it unlikely that basolateral amino acid transport is defective in Hartnup disease.

At a molecular level, the transport characteristics and the epithelial distribution of the Na^+-dependent neutral amino acid transport system B^o (see Specificity and Classification of Amino Acid Transport Systems; Table 1, Fig. 1) fit with defects in Hartnup disease being secondary to mutations in this transport system. In addition, Bröer's group identified SLC6A19 in the mouse (see Table 1) [93] as the gene most likely linked to Hartnup disease in humans. Indeed, in 2004, two groups cloned the human *SLC6A19* gene from chromosome 5p15 and found several mutations in this gene in British, Japanese, and Australian patients with Hartnup disease [94, 95]. To date, a total of 21 mutations have been identified causing Hartnup disease [90, 96]. Interestingly, most of the probands with Hartnup disease analyzed are compound heterozygotes for *SLC6A19* mutations [97].

The presence of alternative transport pathways for neutral amino acids in the renal tubule explains why the defect in the broad-specificity neutral amino acid transport in Hartnup disease results in only partial transport defects for some amino acids (e.g., phenylalanine) [16]. These alternative systems, as well as the oligopeptide-biased carriers in the kidney and intestine, are responsible for normal plasma amino acid levels and lack of symptoms in most patients [98]. Scriver et al. posit that Hartnup disease has multifactorial pathogenesis, and only patients with genetic mutations resulting in low plasma amino acid levels and impaired tryptophan metabolism develop symptoms [98]. Moreover, given the phenotypic heterogeneity of patients with Hartnup disorder [96], it is likely that other mutations (or polymorphisms) in other genes encoding for transport systems for neutral amino acids will be identified in the near future. Furthermore, transport and expression studies in *Xenopus* oocytes [99, 100] suggest that the differences in how mutant B^0AT1/SLC6A19 transporters associate with partner proteins collectrin (Tmem27) and angiotensin-converting enzyme 2 (ACE2) in the kidney or intestine, respectively, likely contribute to the phenotypic heterogeneity of human Hartnup disorder [90, 101].

Patients with Hartnup disease respond well to oral therapy with nicotinamide at 40–100 mg/day [90]. Also, oral administration of tryptophan ethylester, a lipid-soluble form of tryptophan, has been shown to increase serum tryptophan levels and reverse clinical symptoms in patients with Hartnup disease [102].

Methioninuria Methioninuria has been reported in two case reports of patients with isolated increased urinary excretion of methionine and its metabolic breakdown products [103, 104]. These patients had malodorous urine, edema, episodic hyperventilation, seizures, and mental retardation. The underlying defect appears to be abnormal transport of methionine in the kidney and in the intestine. Patients were noted to have high levels of α-hydroxybutyric acid, a bacterial degradation product of unabsorbed intestinal methionine, in the urine. This organic acid could be responsible for the neurologic manifestations observed in these patients. Low-methionine diet resulted in significant clinical improvement. While methionine shares a broad-spectrum amino acid transport system with other neutral amino acids, it is not hyperexcreted in Hartnup disease; this provides evidence that a specific transport pathway for methionine exists in renal and intestinal epithelium.

Histidinuria Isolated histidinuria has been reported in two siblings [105] and two other patients [106, 107]. All were severely mentally retarded. Investigations revealed impaired transport of histidine in the kidney and intestine. Parents of the two affected siblings had normal urinary histidine excretion under normal conditions, but hyperhistidinuria after an oral histidine load. The mode of inheritance of this disease is uncertain, but autosomal-recessive inheritance has been suggested [16]. Although *in vitro* studies have shown that the broad-specificity transporter of neutral amino acids is the major carrier for histidine, fractional excretion rates above 50% have been observed for this amino acid in Hartnup disease [16]; taken with the reports on isolated histidinuria, a specific histidine carrier likely operates in the renal tubule and the intestine.

Iminoaciduria and Glycinuria

Two disorders belong to this group of aminoacidurias: iminoglycinuria and isolated glycinuria.

Iminoglycinuria Iminoglycinuria is an autosomal-recessive membrane transport defect characterized by excretion of excessive amounts of proline, hydroxyproline, and glycine in the urine [108]. Iminoglycinuria is a benign condition with an estimated incidence of 1:15,000 live births [108, 109]. It has been postulated that the shared, brush-border membrane-bound, group-specific transport pathway for imino acids and glycine (PAT; see Specificity and Classification of Amino

Acid Transport Systems; Table 1, Fig. 1) is most likely defective in this condition [108, 110]. The selective glycine-specific (Gly) and imino acid-specific (Imino) transport systems are thought to be affected as well. However, differential function of the selective transporters could account for the normal plasma levels of glycine and imino acids occasionally observed in iminoglycinuria. In addition, immaturity of these selective transporters in normal infant tubules is responsible for neonatal physiologic iminoglycinuria [110]. The lack of function of the two selective transporters in infants with iminoglycinuria, who then also lack the shared transporters, leads to fractional excretion values for glycine and proline approaching 100%, which decline after the first months of life [16]. Defective intestinal transport of proline has been found in some patients with iminoglycinuria [108]. Together, the differential diagnosis of iminoglycinuria includes neonatal iminoglycinuria, Fanconi syndrome in which generalized aminoaciduria occurs, and hyperprolinemia, an inborn error of proline metabolism.

At a molecular level, iminoglycinuria appears to be a genetically and pathophysiologically heterogenous condition with several mutant alleles [16, 108], as is supported by several observations. First, obligate heterozygotes may have isolated hyperglycinuria or may have normal urinary amino acid excretion [111]. Second, some homozygous patients have an intestinal transport defect, while others do not. Third, a variant exists with normal transport maximum (Tm) for proline but with a defect affecting glycine transport capacity more than proline, indicating a K_m defect [112] (see below). In 2008, Bröer and colleagues found that combinations of mutations and polymorphisms in PAT2/*SLC36A2*, XT2/*SLC6A18*, and IMINO/*SLC6A20* (the molecular correlates of PAT, Gly, and Imino, respectively; Table 1) cause iminoglycinuria [113]. The major gene involved in patients that are homozygous for the disease is *SLC36A2*, whereas other patients have mutations and polymorphisms in the genes encoding *SLC6A18* and *SLC6A20* that result in variable renal or renal/intestinal phenotypes and/or in iminoglycinuria or glycinuria phenotypes [113].

Isolated Glycinuria Isolated glycinuria without iminoaciduria has been reported in two families [112, 114]. Some suggest that this condition results from mutations in the specific high-affinity carrier for glycine, namely, XT2/*SLC6A18*, which is expressed in the brush-border membrane of the late proximal tubule (Table 1) [16]. Others have suggested that hyperglycinuria represents a defect in the shared, imino acid-glycine transport system, PAT2/*SLC36A2*, affecting affinity rather than capacity of the system (K_m variant) [25, 113]. Because the condition appears to be inherited as an autosomal-dominant condition, it is also possible that these patients are heterozygotes for the iminoglycinuria allele. Glycinuria has also been reported in association with glycosuria, which is referred to as glycoglycinuria [115]. Together, the genetic defect underlying isolated glycinuria remains unclear.

Dicarboxylic Aminoaciduria

Dicarboxylic aminoaciduria, including selective urinary hyperexcretion of the acidic amino acids glutamate and aspartate, was first reported in two children [116, 117]. One of the children also had impaired intestinal absorption of these amino acids. One patient also developed hypoglycemia that corrected when treated with glutamate and aspartate [116]. This hypoglycemia likely resulted from impaired gluconeogenesis from reduced levels of these gluconeogenic amino acids. This condition is inherited in an autosomal-recessive fashion and appears to be mostly benign; screening in a French-Canadian population revealed a large number of healthy individuals with hyperdicarboxylic aminoaciduria and an incidence of 1: 35,000 live births [118].

Many have long thought that the pathogenic mechanism underlying the disease is a defect in the dicarboxylic amino acid transport system, X^-_{AG}, located in the renal brush-border membrane. Indeed, mutations in the human gene encoding the Na^+/H^+- and K^+-dependent glutamate/aspartate transporter, EAAC1 (*SLC1A1*; EAAT3), that is expressed in the luminal membrane of the late proximal tubule (Table 1; Fig. 1) were later shown to cause dicarboxylic aminoaciduria [119]. Surprisingly, although EAAC1 is widely expressed in the brain and functions as an important neurotransmitter, there is little evidence to suggest that dicarboxylic aminoaciduria leads to significant neural impairment in humans, other than an association with obsessive-compulsive behavior [25, 119]. Unexpectedly, decreased uptake of anionic amino acids has also been observed in cultured skin fibroblasts from affected patients [120].

β-Aminoaciduria

No inborn error of the luminal membrane-bound, Na^+- and Cl^--dependent transport systems that carry taurine/β-alanine (TAUT; *SLC6A6*) and betaine/GABA (BGT1; *SLC6A12*) has been reported in humans. Impaired taurine transport, however, has been detected in the inbred mouse strain C57BL/6J [121]. Studies using kidney slices [121] and isolated basolateral membrane vesicles [122] have localized the transport defect to the basolateral membrane of the proximal tubular epithelium. Similar to the defect in LPI, impaired taurine exit across the basolateral membrane and backflux from cell to lumen may underlie the hypertaurinuria observed in C57BL/6J mice [121, 122]. A transporter mediating transport of taurine across the basolateral membrane has yet to be identified.

Glycosuria

General Characteristics of Renal Glucose Transport

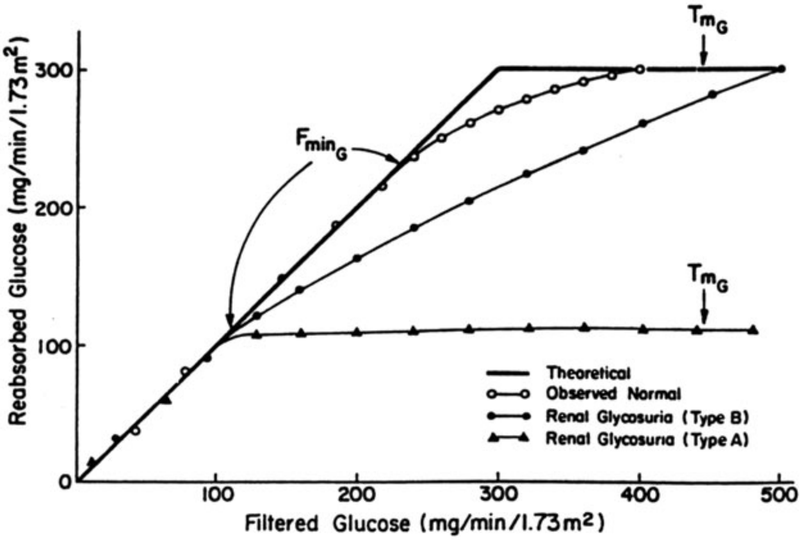
Under normal conditions, reabsorption of filtered glucose by the renal tubule is nearly 100% with less than 0.05% of the filtered glucose load appearing in human urine [123]; 90% of filtered glucose is reabsorbed in the proximal convoluted tubule, and the rest is reclaimed in the proximal straight tubule [123, 124]. No measurable glucose reabsorption occurs distal to the proximal tubule.

Reabsorption of glucose across the proximal tubular brush-border membrane occurs by an active, carrier-mediated, concentrative, Na^+-dependent transport process [123, 124]. The Na^+ electrochemical gradient driving glucose transport across the brush-border membrane is maintained by the basolateral membrane-bound Na^+-K^+-ATPase that transports Na^+ out and K^+ into the cell. Na^+-glucose cotransport across the luminal membrane is electrogenic-positive and phlorizin-sensitive. Glucose exit from the cell occurs by Na^+-independent, facilitated diffusion down the glucose concentration gradient [124]. This diffusional exit of glucose is mediated by a carrier that is distinct from that found at the luminal membrane surface (see later).

The mammalian kidney has a limited capacity to reabsorb D-glucose [123, 125]. As plasma glucose concentration progressively rises, glucose reabsorption increases linearly until reaching a maximum value (Fig. 4). Beyond this maximum rate of glucose reabsorption, further increases in filtered D-glucose load lead to glycosuria. As shown in Fig. 4, the glucose titration curve reveals the minimum threshold (F_{minG}) of glucose transport, defined as the filtered glucose load at which 1 mg of glucose per minute appears in the urine, and the tubular maximum for glucose reabsorption (Tm_G). Reported Tm_G values for glucose in human adults and children range between 260 and 350 mg/minute per 1.73 m^2 [126, 127], with even lower values in infants [127]. When corrected for the GFR (Tm/GFR), transport maximum value for glucose in humans at all ages is approximately 2.5 mg/mL [127]. The glucose titration curve (Fig. 4) is characterized by a splay, or a rounding of the curve, during the transition from virtually complete reabsorption of filtered D-glucose to complete excretion of excess glucose load. This deviation from the theoretical curve is explained by the nephron functional heterogeneity (the gradual progression from the first to the last nephron to saturate) as well as by the variation in affinity for glucose or K_m values between carriers [125].

Studies in isolated perfused tubules [128] and isolated BBMV from pars convoluta (S1 and S2 segments) and pars recta (S3 segment) of the proximal tubule demonstrate that the kinetic properties and stoichiometric relationships of D-glucose reabsorption change along the length of the nephron [129, 130]. These studies were the first to provide evidence for two Na^+-dependent

Fig. 4 Renal glucose titration curves. Theoretical and observed normal curves are compared to abnormal curves observed in type A and type B renal glycosuria. Tm_G, maximum rate for glucose reabsorption; F_{minG}, minimum threshold. (Reprinted with permission from ref. #125)



transport mechanisms, a low-affinity/high-capacity cotransport system in the early proximal tubule and a high-affinity/low-capacity system in the late proximal tubule. The Na^+-glucose coupling ratio was found to be 1:1 in the convoluted proximal tubule and 2:1 in the straight proximal tubule [129, 130]. The efficiency of a coupled carrier system increases with the power of the stoichiometry [124, 130]. Whereas the early proximal tubule glucose transporter is responsible for the reabsorption of the bulk of filtered D-glucose from the tubular lumen, the late proximal tubule glucose transporter is responsible for the removal of all but the last traces of glucose from the urine [130]. The arrangement of transporters in series along the proximal tubule enables the kidney to reabsorb glucose from the urine in a more energy-efficient mechanism than could be achieved by either of the cotransporters acting alone.

Molecular Biology of Na^+-Glucose Cotransporters

Considerable progress has been made in the past two decades in elucidating the molecular structure of membrane proteins responsible for sugar transport, including the Na^+-glucose cotransporter [123, 131, 132, 133]. Hediger et al. used expression cloning in *Xenopus* oocytes to clone and sequence the first ever identified Na^+-dependent D-glucose transporter from rabbit [21] and human [134] intestine. Subsequently, molecular analysis revealed that rabbit intestinal and renal Na^+-glucose cotransporters are nearly identical. The transporter was later termed SGLT1 and the gene encoding it, *SLC5A1* [131]. The Na^+-glucose cotransport proteins belong to the SLC5 family of sodium cotransport proteins. Included in the SLC5 family are 12 human proteins, of which 6 are Na^+-glucose cotransporters (SGLTs) that are expressed in the kidney and other organs [132, 133]. The SGLT1 (*SLC5A1*) transporter, the prototype of the SGLT group, consists of 664 amino acids and has 14 membrane-spanning domains. In humans, the SGLT1 gene maps to chromosome 22q11.2 [135]. Expression studies by Pajor et al. [136] and Lee et al. [137] have provided evidence that the renal Na^+-glucose cotransporter SGLT1 is the high-affinity/low-capacity transporter found predominantly in the straight proximal tubule (S3 segment). Stoichiometry studies were consistent with a Na^+-to-glucose coupling ratio of 2:1 [137]. The SGLT1 (*SLC5A1*) gene has been implicated in hereditary glucose-galactose malabsorption (see Hereditary Glycosuria).

A second, low-affinity Na^+-glucose cotransporter named SGLT2 (*SLC5A2*) was later isolated from rat and human kidney [138]. The amino acid sequence of SGLT2 is 59% identical to that of SGLT1. SGLT2 has a Na^+-glucose coupling ratio of 1:1 and is strongly expressed in proximal tubule early (S1 and S2) segments [138]. Human SGLT2 gene was mapped to chromosome 16p11.2 close to the centromere [139]. Clinical, physiologic, and molecular data indicate that the SGLT2 gene, *SLC5A2*, is responsible for familial renal glycosuria (see Hereditary Glycosuria).

Molecular Biology of Facilitative Glucose Transporters

Glucose concentrated inside tubular epithelial cells flows to the interstitium down its concentration gradient through facilitative glucose transporters located in the basolateral membrane [123, 140]. The basolateral membrane glucose transporters belong to the SLC2 family of Na^+-independent facilitative hexose transporters, which includes 14 glucose transporters GLUT1–14 [140, 141]. The human erythrocyte glucose transporter GLUT1 (*SLC2A1*) was the first facilitative glucose transporter to be cloned and sequenced. It has 12 membrane-spanning domains and is the most ubiquitously distributed among the various isoforms [140, 141]. Studies using specific anti-GLUT1 antibodies have detected this high-affinity transporter in the kidney [142, 143], in the basolateral membrane of cells of the proximal straight tubule (S3 segment). GLUT2 (*SLC2A2*), a low-affinity transporter, is the predominant facilitative glucose transporter in hepatocytes and in the basolateral membrane of intestinal and renal tubular cells [142, 143]. In the kidney, GLUT2 is present only in the basolateral membrane of cells in the proximal convoluted tubule (S1 and S2 segments) [142, 143]. *SLC2A2* has been identified as the mutated gene in Fanconi-Bickel syndrome (see Hereditary Glycosuria).

In summary, transepithelial glucose transport in the proximal tubule occurs by two different pairs of apical Na^+-dependent and basolateral Na^+-independent glucose transporters [123, 140] (Fig. 5). A luminal Na^+-dependent, low-affinity/high-capacity glucose transporter designated SGLT2/*SLC5A2* and a basolateral Na^+-independent, low-affinity GLUT2/*SLC2A2* are responsible for the bulk of glucose reabsorption in the early part of the proximal tubule. A luminal Na^+-dependent, high-affinity/low-capacity SGLT1/*SLC5A1*, coupled with the basolateral Na^+-independent, high-affinity GLUT1/*SLC2A1*, reabsorbs the remaining low concentration of glucose in the late part of the proximal tubule.

Hereditary Glycosuria

Familial Renal Glycosuria

Familial renal glycosuria (OMIM# 233100) is characterized by variable amounts of glucose excreted in the urine despite normal concentrations of blood glucose [144]. The renal defect is specific for glucose and there is no increase in the urinary excretion of other sugars. Renal glycosuria is a benign condition without symptoms or physical consequences except during pregnancy or prolonged starvation, when dehydration and ketosis may develop [144]. Metabolism, storage, and use of carbohydrates as well as insulin secretion are normal. Diagnosis usually is done on routine urine analysis often during well visits. The distinction between renal glycosuria and diabetes mellitus is made with a fasting blood glucose level and a glucose tolerance test. The inheritance pattern in renal glycosuria is usually autosomal recessive, although glycosuria has been noted in some heterozygotes, leading some investigators to postulate a dominant inheritance [144, 145]. Renal glycosuria is not associated with impaired D-glucose transport in the intestinal epithelium [126]. The underlying pathogenic mechanism appears to be an isolated, selective defect in proximal tubular glucose transport.

Familial renal glycosuria is a heterogenous condition. Analysis of renal titration curves for glucose reabsorption reveals two types of renal glycosuria [125, 144] (Fig. 4). In type A, or classic renal glycosuria, minimal glucose threshold (F_{minG}) and maximum rate of glucose reabsorption (Tm_G) are reduced. In type B, F_{minG} is reduced, while Tm_G is normal but has an increased splay. It has been suggested that the type A mutation reflects reduction in the capacity of the glucose transport system, which might arise from a uniform defect in all

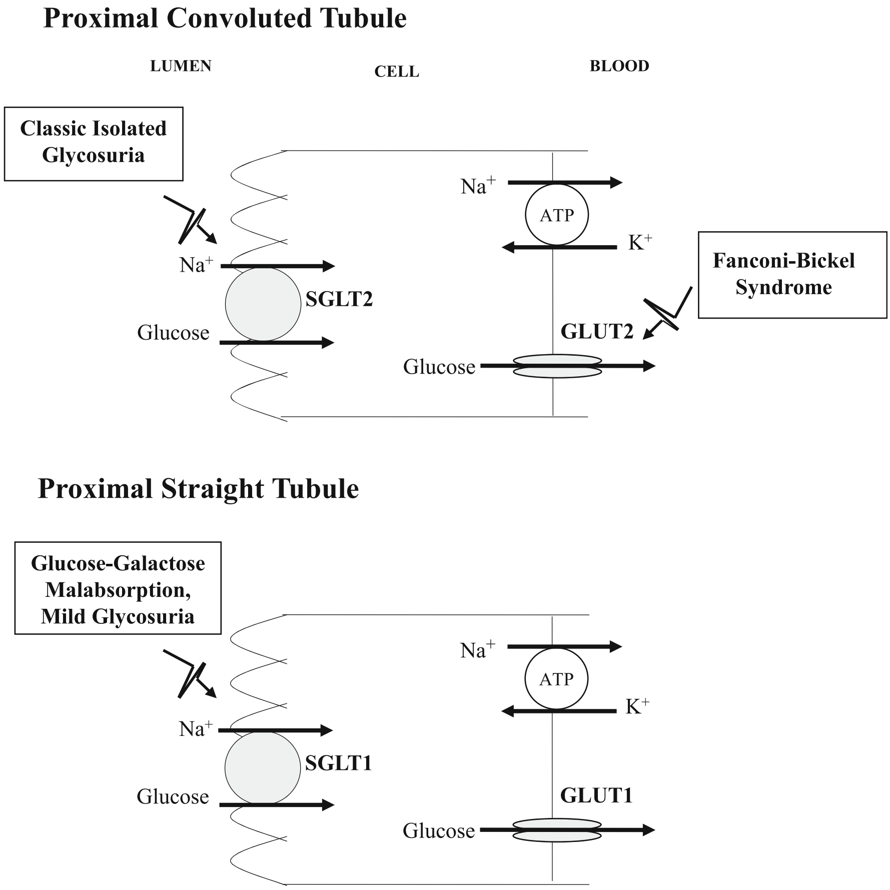

Proximal Convoluted Tubule
LUMEN
CELL
BLOOD
Classic Isolated Glycosuria
Na+
Glucose
SGLT2
Na+
ATP
K+
Fanconi-Bickel Syndrome
GLUT2
Glucose
Proximal Straight Tubule
Glucose-Galactose Malabsorption, Mild Glycosuria
Na+
Glucose
SGLT1
Na+
ATP
K+
GLUT1
Glucose

Fig. 5 Schematic model for the distribution of luminal and basolateral glucose transporters in renal tubular epithelium. Orientation permits net reabsorption from the lumen to interstitium. Hereditary glycosurias caused by defects in these transporters are depicted. See text for details

nephrons, and type B reflects a decrease in the affinity of the transport system, which might also be a consequence of nephron heterogeneity [126, 146]. A third type of glycosuria, termed type O, was described by Oemar et al. [147]. In this rare latter condition, tubular reabsorption of glucose is virtually absent, and all glucose filtered is excreted in the urine.

Regarding genetics, in 2002, the SGLT2 gene, *SLC5A2*, was first established as the mutated gene in familial isolated glycosuria [148] (Fig. 5). Subsequent studies have found nearly 30 *SLC5A2* mutations in patients with familial renal glycosuria [133, 149, 150]. These include missense, nonsense, and frameshift mutations. Interestingly, some *SLC5A2* mutations may result in renal glycosuria accompanied by generalized aminoaciduria [151]. Renal glucose wasting associated with generalized aminoaciduria is also a feature of maturity-onset diabetes of the young type 3 (MODY 3) caused by a mutation in the hepatocyte nuclear factor-1 alpha (HNF-1α) gene [152]. HNF-1α acts as a regulator of both transcription of *SLC5A2* [153] and expression of collectrin, important for neutral amino acid transport [154] (see Hartnup Disease). Hence, mutations or polymorphisms in HNF-1α may explain the link between glycosuria and aminoaciduria observed together in some cases of hereditary renal glucosuria and in MODY 3.

Unlike isolated renal glycosuria, which is a benign condition, intestinal glucose-galactose malabsorption is a potentially lethal disease. Glucose-galactose malabsorption (OMIM# 182380) is an autosomal-recessive disease characterized by a neonatal onset of severe watery diarrhea that may result in death unless glucose and galactose are removed from the diet [144]. Studies using jejunal biopsy specimens from affected patients have demonstrated a defect in intestinal Na^+-dependent glucose transport [155]. In 1991, molecular genetic studies in two sisters afflicted with glucose-galactose malabsorption revealed a missense mutation that causes an amino acid change in residue 28 from aspartate to asparagine in the intestinal brush-border SGLT1 Na^+-glucose cotransporter [156]. Thus, glucose-galactose malabsorption was the first reported disease that is caused by a mutation in a membrane transport protein. To date, more than 40 mutations in SGLT1 have been identified in patients with glucose-galactose malabsorption [133]. Mutant proteins cause a marked reduction in Na^+-glucose transport activity, which, in most cases, is due to missorting of the protein to the cell surface [133, 144]. Patients with glucose-galactose malabsorption have a mild defect in renal tubular reabsorption of glucose, with normal Tm for glucose but decreased minimal threshold (F_{minG}) [144, 155]. In contrast, patients with renal glycosuria show no defect in intestinal D-glucose absorption. Together, these data revealed that the SGLT1 Na^+-glucose cotransporter affected in glucose-galactose malabsorption is shared between the intestine and the kidney, as previously suggested [136, 137] (see above), while the glucose transporter impaired in glycosuria is only present in the kidney.

In summary, the clinical, physiologic, and molecular data on renal glucose transport to date have now led to the following model of the pathogenesis of familial renal glycosuria [123, 133, 157, 158] (Fig. 5). Defects in the low-affinity/high-capacity, 1 Na^+/1 glucose cotransporter (SGLT2/*SLC5A2*) of the early proximal tubule, which reabsorbs most renal tubular glucose, produce type A or type O classic renal glycosuria but have no effect on glucose absorption in the intestine. Studies in SGLT2-deficient mice [159, 160] have confirmed that SGLT2 mediates the reabsorption of most filtered glucose molecules in the early proximal tubule. This mouse model mimics and explains the glycosuria in patients with SGLT2/*SLC5A2* mutations. In contrast, SGLT1/*SLC5A1*, the high-affinity/low-capacity, 2 Na^+/1 glucose cotransporter of the late proximal tubule, mediates residual glucose reabsorption in the renal tubule and, when defective as in glucose-galactose malabsorption, causes only mild type B renal glycosuria.

Fanconi-Bickel Syndrome

Mutations in the gene encoding GLUT2 (*SLC2A2*), the basolateral membrane-bound, facilitative glucose transporter of the proximal convoluted tubule, are associated with glycosuria in the Fanconi-Bickel syndrome (OMIM# 227810) [161, 162, 163] (Fig. 5). This autosomal-recessive disorder is characterized by hepatorenal glycogen accumulation, fasting hypoglycemia, impaired utilization of glucose and galactose, renal Fanconi syndrome, rickets, and markedly stunted growth [163, 164]. The renal glucose losses are due to transport defects for monosaccharides across the renal basolateral membrane. It is conceivable that this results in glucose accumulation in the cell and glycotoxicity leading to the generalized proximal tubulopathy. To date, approximately 70 different mutations in *SLC2A2* have been detected in patients with Fanconi-Bickel syndrome [164, 165]. The mutations in GLUT2/*SLC2A2* can lead to quite variable clinical and biochemical findings and different degrees of proximal tubular dysfunction [165, 166, 167]. Therapy for patients with Fanconi-Bickel syndrome is targeted toward symptoms and includes stabilization of glucose homeostasis, treatment of rickets, replacement of renal solute losses, and promotion of growth [163].

Cross-References

- ▶ Pediatric Fanconi Syndrome
- ▶ Renal Manifestations of Metabolic Disorders in Children

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Renal Hypophosphatemia

40

Dieter Haffner and Agnes Linglart

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Abstract

Over the last decade, research in the field of genetic disorders resulting in hypophosphatemia has significantly broadened our understanding of phosphate metabolism. X-linked hypophosphatemia is the most common inherited form of rickets, which is caused by renal phosphate wasting. The common denominator for all types of rickets is hypophosphatemia, which leads to an insufficient supply of the mineral to the growing bone. Recent findings on disease mechanisms and the role of fibroblast growth factor 23 (FGF23) in hypophosphatemic diseases have opened up new therapeutic avenues such as FGF23 blockade. We will discuss

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the genetic and clinical features of hypophosphatemic disorders and provide understanding and treatment options based on recent guidelines.

Keywords

X-linked hypophosphatemia · Fibroblast growth factor 23 · Vitamin D · Rickets · Osteomalacia · Burosumab · Nephrocalcinosis · Renal phosphate wasting

Introduction

The etiology of hypophosphatemic disorders has been elucidated over the last decade, although not all puzzles were solved. In 1937, Albright first reported on a patient with rickets and severe hypophosphatemia not responding to high doses of vitamin D. The term vitamin D-resistant rickets was coined, and later the disease was called X-linked hypophosphatemic rickets respective X-linked hypophosphatemia [1].

In recent years, several underlying genes have been identified in different forms of hypophosphatemic rickets [2]. Rickets is a disease of the growth plate, which is why only growing children are affected [3]. While rickets has historically been thought to be a disease of calcium and vitamin D metabolism, there is increasing evidence that rickets is caused by the insufficient availability of phosphate, which is required for normal bone metabolism [4]. In principle, a phosphate deficiency can be caused by inadequate absorption in the intestine or kidneys. The latter situation can be further divided into defects in the tubular reabsorption apparatus and abnormalities of circulating factors that regulate phosphate reabsorption [5]. The discovery of fibroblast growth factor 23 (FGF23) was a major breakthrough in our understanding of hypophosphatemic disorders. FGF23 is a member of the FGF family, which mediates the combined renal tubular defects in phosphate reabsorption and altered vitamin D metabolism observed in many forms of hypophosphatemic rickets including XLH [6].

Nowadays, specific disease-causing mutations in genes involved in the regulation of phosphate homeostasis can be identified in approximately 85% of familial or sporadic cases of hypophosphatemic rickets [7–10]. This chapter deals with the etiology, clinical presentation, differential diagnosis, and management of hypophosphatemic disorders due to a decrease in renal tubular phosphate reabsorption.

Phosphate Homeostasis

Inorganic phosphate (Pi) plays a major role in cellular metabolism and skeletal mineralization. It accounts for about 0.6 and 1% of body weight of a neonate and an adult, respectively. Approximately 85% of total body Pi content is deposited as hydroxyl-apatite [$Ca_5(PO_4)_3OH$] in the skeleton and the teeth, and 14% distributes within the intracellular compartment. Inorganic phosphate is heavily involved in multiple cellular processes including cell membrane function (phospholipids), energy metabolism (ATP), cell signaling (phosphorylation by kinases), and DNA- or RNA-biosynthesis (phosphorylated nucleotides). Only 1% of the total body Pi content is found as a soluble fraction in the extracellular compartment. It contributes to the acid-base buffering capacity of the plasma and of the urine. Thus, if in clinical practice the phosphate concentration in serum or urine is determined, only "the tip of the iceberg" is visible. In addition, circulatory Pi is the central mediator between bone, the parathyroid glands, the intestinal tract, and the kidneys, which are fine-tuned by numerous hormonal signals in order to keep the serum phosphate concentration within close limits.

In adults, skeletal growth is completed and a steady state condition can be assumed. In this case, the serum Pi concentration is determined by the balance between intestinal absorption of phosphate from the diet (16 mg/kg per day), bone-turnover of phosphate in the skeleton (3 mg/kg per day), and urinary phosphate excretion (16 mg/kg per day), (Fig. 1). In children and adolescents, the balance of Pi must be positive to meet the needs of skeletal growth and consolidation. A typical

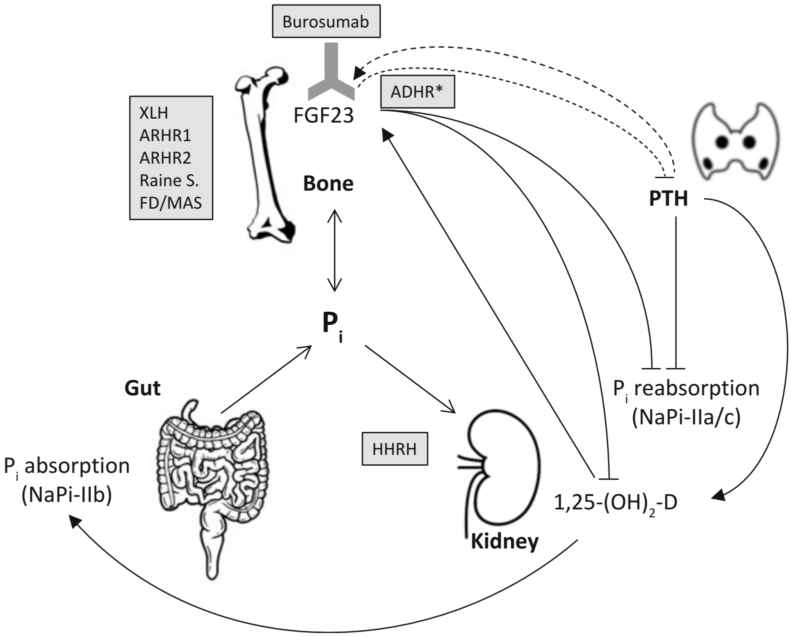


Fig. 1 Regulation of phosphate (P_i) homeostasis. FGF23 and PTH reduce renal tubular phosphate reabsorption via a decrease in apical expression of the sodium-phosphate cotransporters NaPi IIa and NaPi IIc. In contrast to FGF23, which inhibits 1,25(OH)$_2$D synthesis, PTH stimulates 1,25(OH)$_2$D production. 1,25(OH)$_2$D increases intestinal absorption of dietary P_i via increased expression of NaPi IIb and activates FGF23 production. PTH and FGF23 affect each other's production through a negative feedback loop that is not yet fully elucidated. The mode of action of the therapeutic antibody burosumab is indicated. Note, burosumab is currently licensed for treatment of XLH and tumor-induced osteomalacia (TIO), only. The sites of defect of the different genetic forms of hypophosphatemic disorders are given. XLH, X-linked hypophosphatemia; ARHR1/2, autosomal recessive hypophosphatemic rickets 1/2; Raine S., Raine syndrome; FD/MAS, Fibrous dysplasia/McCune-Albright syndrome; HHRH, hereditary hypophosphatemic rickets with hypercalciuria; ADHR, X-linked-dominant hypophosphatemic rickets. *FGF23 protein resistant to degradation

Western diet commonly provides plenty of alimentary Pi, most of which is provided by protein-rich foods such as meat, milk, and eggs. In contrast to plant-derived phosphate in the form of phytate (hexa-phospho-inosite), the animal-derived phosphate is easily absorbed by the intestine, where roughly two-thirds of the ingested phosphate is absorbed. Intestinal Pi absorption in infants is markedly higher than in adults due to the demands of a fast-growing skeleton and can exceed 90% of dietary intake. Absorbed Pi first distributes in the extracellular compartment and then equilibrates with the bone and intracellular compartment. Within the kidney, Pi is freely filtered via the glomerular capillaries and is reabsorbed mainly along the proximal nephrons according to the actual needs of the body. The majority of the transepithelial Pi transport in the intestine and the kidney is mediated by the type II family of sodium-coupled phosphate transporters, i.e., NaPi-IIa (NPT2a; *SLC34A1*), NaPi-IIb (NPT2b; *SLC34A2*), and NaPi-IIc (NPT2c; *SLC34A3*) [11]. NaPi-IIa is localized at the brush border of proximal tubular epithelial cells and accounts for roughly 90% of phosphate reabsorption by the kidneys. Reabsorption of the remaining 10% is accomplished by NaPi-IIc exclusively expressed along proximal tubules of deep nephrons. Their important role in Pi homeostasis is demonstrated by loss-of-function mutations resulting in renal phosphate wasting as noted in the rare syndrome of hypophosphatemic rickets with hypercalcemia (HHRH) [12].

In contrast to NaPi-IIa and NaPi-IIc, NaPi-IIb shows a broader expression pattern including pulmonary alveolar type II cells, where it participates in Pi uptake from the alveolar fluid for surfactant production. *SLC34A2* mutations cause pulmonary alveolar microlithiasis. Patients suffer from the deposition of calcium-phosphate crystals throughout the lungs [13]. In the intestine, NaPi-IIb is expressed in the brush border membrane of enterocytes and mediates absorption of phosphate from food.

Regulators of Phosphate Homeostasis

The amount of intestinal phosphate absorption is highly correlated to the dietary intake. Only 30% of intestinal Pi absorption occurs in a regulated, 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D]-dependent manner. This poor regulation at the uptake level contrasts with the meticulous regulation of renal phosphate excretion. Proximal tubular reabsorption of phosphate, mainly via regulation of expression of NaPi-IIa and NaPi-IIc within proximal tubular brush border membranes, thus plays a key role in maintaining serum phosphate homeostasis. The amount of phosphate reabsorption by the kidneys is tightly regulated primarily by dietary Pi intake, parathyroid hormone (PTH), and fibroblast growth factor 23 (FGF23). Other factors such as insulin, growth hormone and its mediator insulin-like growth factor 1, and possibly FGF7 also affect renal phosphate handling, but their actions are less well understood [14].

Dietary Pi intake directly affects the amount of renal phosphate reabsorption. An increase or decrease in dietary Pi induces an increase or decrease in renal Pi excretion independent of vitamin D, PTH, or FGF23. Part of this effect could be explained by a phosphate-responsive element in the promoter of the *SLC34A1* gene [15].

Parathyroid hormone is an important hormonal regulator of proximal tubular Pi reabsorption. PTH synthesis and secretion are upregulated by low serum calcium and elevated serum phosphate levels, and downregulated by elevated serum calcium and 1,25$(OH)_2$D levels. Its binding to proximal tubular PTH receptors results in inhibition of NaPi-cotransport via mechanisms involving rapid clearance of NaPi-IIa from the tubular epithelial brush border membrane [16]. In addition, PTH also stimulates the synthesis of 1,25-$(OH)_2$D. The net effect of these actions is an increase in serum calcium levels and a decrease in serum phosphate levels.

Fibroblast Growth Factor 23 is a glycoprotein that is mainly synthesized in osteocytes and osteoblasts. The expression of FGF23 is induced by elevated serum phosphate and 1,25$(OH)_2$D levels. In the presence of Klotho, a membrane-bound protein with ß-glucuronidase activity, FGF23 binds with high affinity to the FGF receptor FGFR1 [14]. Klotho/FGFR1-mediated renal effects of FGF23 lead to an inhibition of proximal tubular Pi reabsorption and 1,25$(OH)_2$D synthesis. The net effect is thus a reduction in serum phosphate and 1,25-$(OH)_2$D levels, which may lead to hypocalcemia (Fig. 1).

Hypophosphatemic Disorders

Clinical, Biochemical, and Radiographic Manifestations Including Differential Diagnosis

Children with chronic hypophosphatemia experience growth retardation and rickets. Rickets is defined as an abnormality of differentiation and maturation of chondrocytes resulting in disorganized chondrocyte columns, and lack of mineralization of the growth plate. It is associated with decreased serum calcium and/or phosphate levels, leading primarily to widening and delayed mineralization of growth plates. Rickets is always associated with osteomalacia, i.e., a deficient mineralization of the bone matrix [17].

Bone deformities are the hallmark of rickets. Affected children present with progressive bowing of the femur and tibia. The site and type of deformity of the extremities depend upon the age of the child and the weight-bearing patterns of the limbs. Deformities of the forearms and posterior bowing of the distal tibia are more common in infants, whereas an exaggeration of the normal physiological bowing of the legs (genu varum) is a characteristic finding in the toddler who has started to walk. In walking children, valgus deformities of the legs or a windswept deformity (valgus deformity of one leg and varus deformity of the other) may be apparent. Deformities of the arms are virtually absent in hypophosphatemic rickets (Fig. 2). Palpable and visible knobby enlargements of the extremities of the long bones and osteochondral junctions (the "rachitic rosary") are the clinical manifestation of the disorganized hypertrophic growth plate. Because of the abnormal mineralization of the growth plate, growth is inevitably affected; in advanced forms, the growth may be stunted.

Severe rickets may also include delayed closure of the fontanelles, parietal and frontal bossing, craniotabes (soft skull bones), and craniosynostosis (Fig. 3A**).** Muscles are also affected with decreased muscle tone, leading to delayed achievement of motor milestones and a waddling gait [17–19]. In children, signs or severity of rickets should be assessed at each visit including measurement of intercondylar and/or intermalleolar distance (Fig. 4), as well as height, and growth velocity. Endodontic abscesses in teeth that seem intact are the major dental features of XLH in children (Fig. 3B**).** Usually, teeth are devoid of caries, history of trauma, or periodontal disease [20].

Radiologic changes of rickets are best visualized on radiographs at the growth plate of rapidly growing bones, i.e., the distal ulna, metaphyses above and below the knees, and at the ankles. Increased height of the physis is common to all forms of rickets. Widening of the epiphyseal plate and loss of definition of the zone of provisional calcification at the epiphyseal/metaphyseal interface are the early signs of rickets (Figure 5A). As the disease progresses, disorganization of the growth plate becomes more apparent with cupping, splaying, formation of cortical spurs, and stippling. The appearance of the epiphyseal bone centers may be delayed, or they may be small, osteopenic, and ill-defined. Deformities of the shafts of the long bones are often present. In severely affected adolescents, pathological fractures and Looser's zones, narrow radiolucent lines 2–5 mm in width with sclerotic borders also known as Milkman pseudofractures, may be noted.

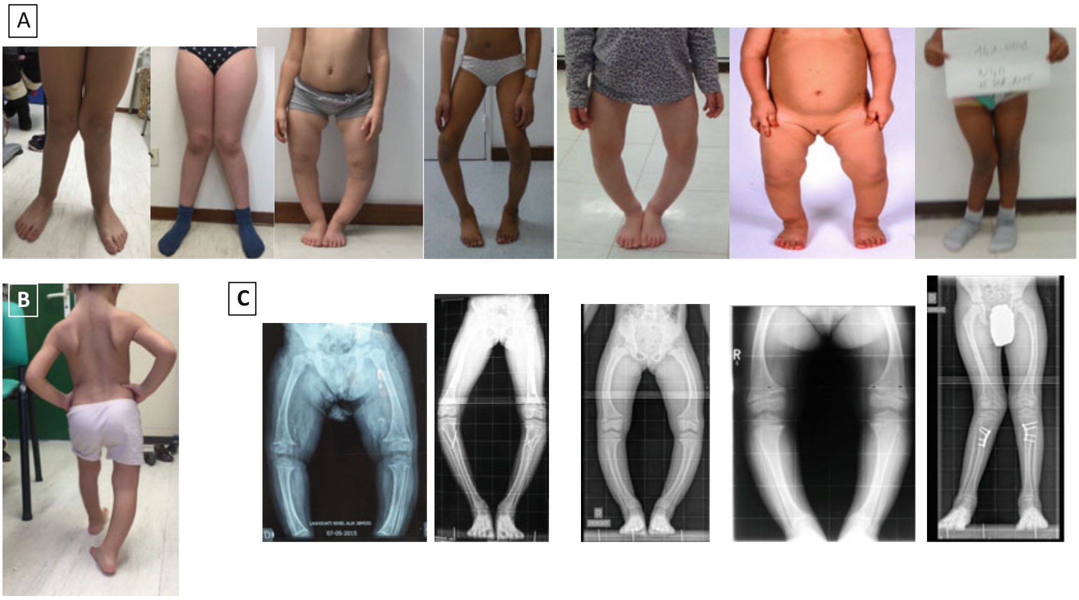


Fig. 2 Clinical and radiographic features of XLH. Photograph (upper **panel A**) and corresponding radiographs of the lower extremities (lower **panel C**) of children affected with X-linked hypophosphatemia (XLH). The patients show disproportionate short stature with different shapes of bowed legs (*genu valgum* [first two panels on the left], *genu varum*, and windswept [last panel on the right]). On **panel B**, the picture displays the typical waddling gait. The radiographs reveal severe leg bowing, partial fraying, and irregularity of the distal femoral and proximal tibial growth plates. Note the lack of bone resorption features on X-rays

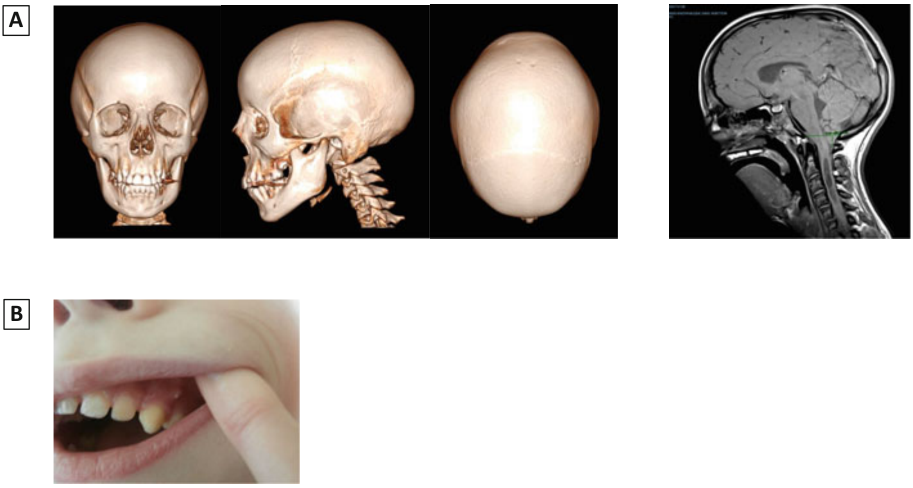


Fig. 3 Craniosynostosis in a child with XLH. (**a**) Typical pansynostosis; Chiari 1 malformation and syringomyelia. (**b**) Dental abscess on an apparently healthy tooth

Interestingly, in contrast to rickets due to an abnormal vitamin D intake or metabolism, where intracortical bone resorption with tunneling is a typical radiological finding, the cortical bone in children with hypophosphatemic rickets has a normal appearance or may even appear as thickened without signs of bone resorption [21, 22].

The rickets severity can be evaluated using the Rickets Severity Score (RSS), a quantitative scale developed for nutritional rickets by Tom Thacher. RSS is based on the degree of metaphyseal fraying and concavity, and the proportion of the growth plate affected at the wrists and knees [23].

MRI of the knee may be used as a tool to image the physis and feature the rickets in pediatric patients, allowing to minimize ionizing radiation in children and comply with keeping doses “as low as reasonably achievable” (ALARA) – or even “as low as diagnostically acceptable” (ALADA) (Figure 5B). MRI of the knees depicts rachitic changes in cartilage with an excellent resolution and three-dimensional acquisition, allowing a precise evaluation of physeal widening and transverse extent [21]. Radiographs and MRI of the knees allow precise monitoring of ricketic lesions during pharmacological treatment (Fig. 6).

Elevated alkaline phosphatase (ALP) activity confirms the diagnosis of rickets in patients with bone deformities and enlargement of the physes. It excludes all the differential diagnoses of abnormal metaphyses and bone deformities including Blount disease and metaphyseal dysplasia. It may also exclude hypophosphatasia in young patients with “ricketic” bone changes, yet elevated levels of calcium and/or phosphate. ALP levels tend to be moderately elevated in hypophosphatemic rickets (1–3 times the upper level of normal) in contrast with nutritional and hypocalcemic rickets with elevated PTH where ALP values are often greater (up to 10 ULN or more). Beside supporting the diagnosis of rickets, ALP is an excellent marker of disease activity and is, therefore, used as a tool to monitor treatment [18].

Low serum phosphate levels are found in almost all causes of rickets (Table 1**)** [4]. It is therefore of utmost importance to identify the pathological mechanism that leads to the chronic insufficient supply of phosphate, i.e., (i) high PTH activity, (ii) inadequate phosphate absorption from the gut, or (iii) renal phosphate wasting. The latter may be due to either tubular defects or high-circulating FGF23 levels (Fig. 7). The tubular reabsorption of phosphate (TRP) and the maximal tubular reabsorption of phosphate per glomerular filtration rate (TmP/GFR) are usually decreased, in all types of rickets, but are more pronounced in renal phosphate wasting types. An important pitfall to know is that in patients with insufficient intake or absorption of phosphate from the gut, TmP/GFR might be falsely low

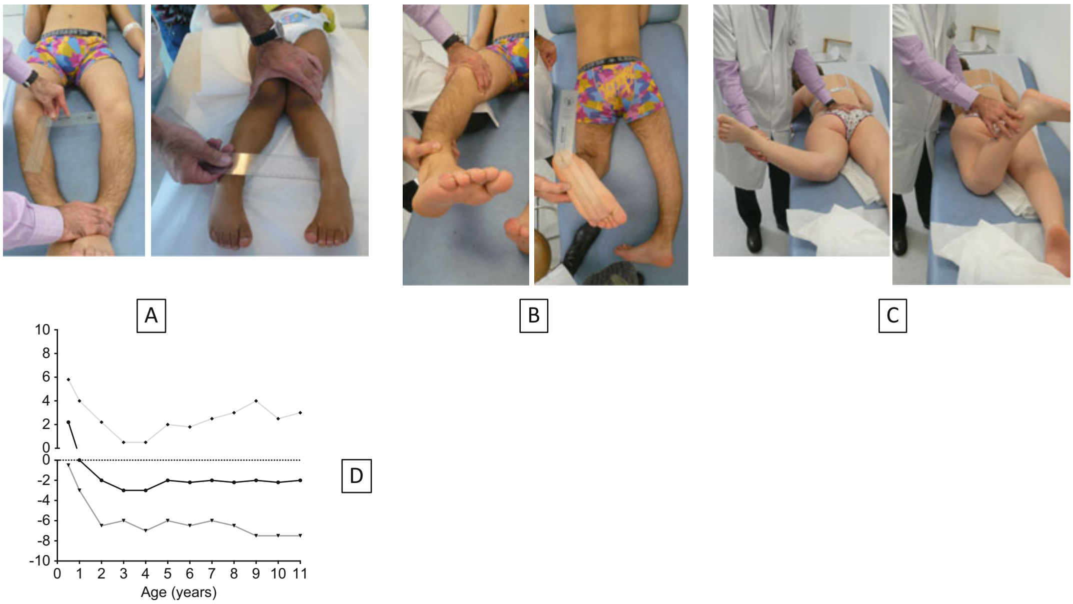


Fig. 4 Clinical assessment of bone deformities of lower limbs. (**a**) Illustration of the process of measuring the medial intermalleolar distance, (**b**) medial femoral intercondylar distance, and (**c**) medial tibial torsion and long bone torsion. (**d**) Physiological distance between knees and ankles in children. The upper part of the graph represents the 2 SD intercondylar distance (ICD, positive) and the lower part the 2 SD. intermalleolar distance (IMD, negative) and the mean values are represented in the middle of the graph. Note, measurement of IMD and ICD cannot replace a regular careful orthopedic assessment due to high complexity of leg deformities (e.g., torsion of limbs) in many patients [93]

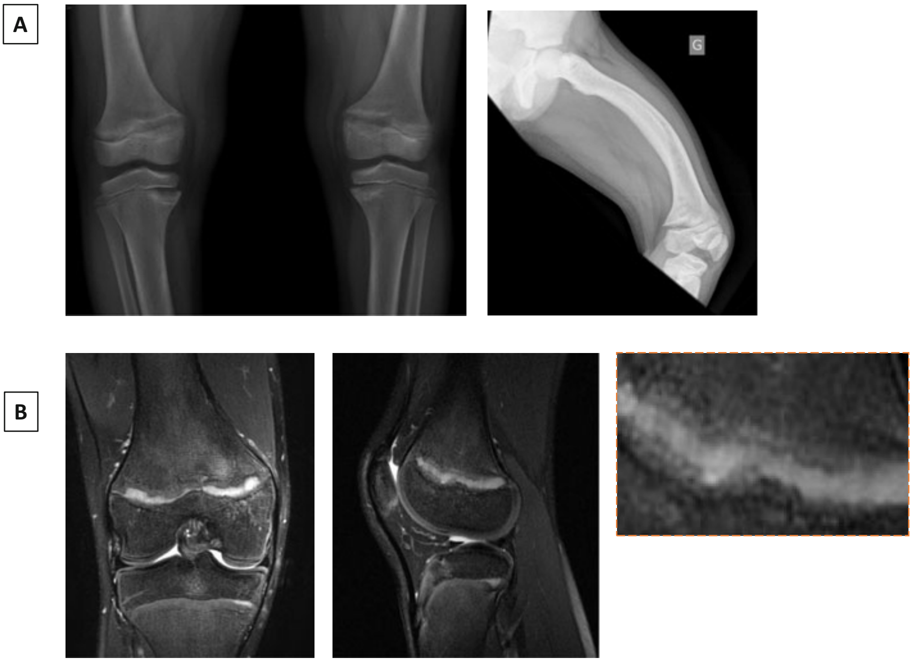


Fig. 5 Radiographic features of XLH. (**a**) Radiographs: Enlarged metaphyses with blurred limits; bowed femur and thick cortices in XLH. (**b**) Knee MRI. Increased physes spanning about 75% of the growth plate surface; the growth plate layers are visible on MRI

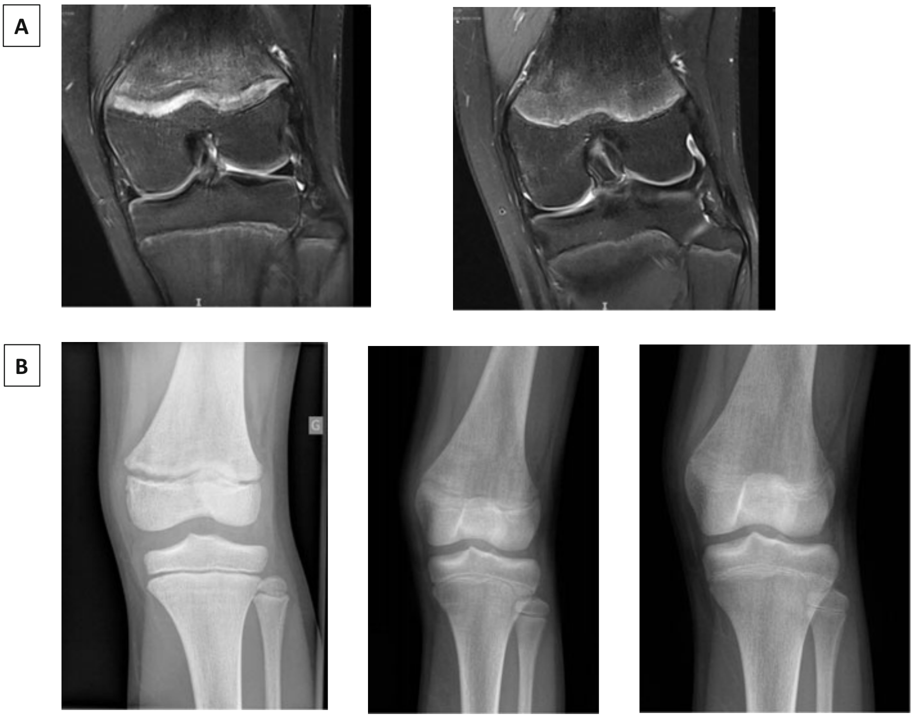


Fig. 6 Evolution of ricketic changes in children treated with burosumab. (**a**) Knee MRI. Increased physes spanning about 90% of the growth plate surface, in a child who received conventional therapy during 7 years; improvement after 1 year of burosumab on the left panel. (**b**) Radiographs: Enlarged metaphyses in a young girl (9 years old) treated with conventional therapy for 6 years; improvement after 2 and 3 years of burosumab therapy (middle and left panels)

when serum phosphate levels have not been restored to normal. This can usually be assumed in the presence of low urinary phosphate levels. In such cases, TmP/GFR should only be calculated after phosphate supplementation when serum and urine phosphate concentrations are raised.

The PTH concentrations are usually normal or modestly elevated in phosphopenic rickets. In most phosphopenic rickets, serum concentrations of 1,25(OH)2D may be low or inappropriately normal (in view of the ambient hypophosphatemia, which should trigger an increased production of the metabolite).

After exclusion of other causes of hypophosphatemia, and characterization of hypophosphatemia due to renal phosphate wasting, the measure of intact FGF23 contributes to the diagnosis of hypophosphatemic rickets (Fig. 7). Reference values for intact [24] and c-term FGF23 [25] in children are available but vary considerably according to the assay used. Therefore, in clinical practice the result in an individual patient should always be compared to reference values obtained using the same assay. FGF23 levels are also influenced by other factors, in particular phosphate intake and vitamin D therapy. Therefore, FGF23 levels are most informative in untreated patients. Finally, low normal FGF23 levels do not exclude FGF23-driven hypophosphatemia but should be interpreted as inappropriately normal in the setting of hypophosphatemia.

Table 1 summarizes the molecular genetic and biochemical characteristics of inherited or acquired causes of phosphopenic rickets in comparison to calcipenic rickets. The biochemical workup in children affected with hypophosphatemic rickets should initially include the measurement of phosphate, calcium, ionized calcium, creatinine, PTH, 25(OH)D, $1,25(OH)_2D$, FGF23, and an assessment of the renal tubular function, e.g., urinary potassium, sodium,

Table 1 Molecular genetic and biochemical characteristics of inherited or acquired causes of phosphopenic rickets in comparison to calcipenic rickets

Disorder (abbreviation; OMIM#)	Gene (location)	Ca	P	ALP	U_{Ca}	U_P	TmP/ GFR	FGF23	PTH	25 (OH) D^a	1,25 $(OH)_2D$	Pathogenesis
Rickets and/or osteomalacia with high PTH levels (calcipenic rickets)												
Nutritional rickets (vitamin D and/or calcium deficiency)	NA	N, ↓	N, ↓	↑↑↑	↓	Varies	↓	N	↑↑↑	↓↓, N	Varies	Vitamin D deficiency
Vitamin D-dependent rickets type 1A (VDDR1A; OMIM#264700)	*CYP27B1* (12q14.1)	↓	N, ↓	↑↑↑	↓	Varies	↓	N, ↓	↑↑↑	N	↓	Impaired synthesis of 1,25 $(OH)_2D$
Vitamin D-dependent rickets type 1B (VDDR1B; OMIM#600081)	*CYP2R1* (11p15.2)	↓	N, ↓	↑↑↑	↓	Varies	↓	N	↑↑↑	↓↓	Varies	Impaired synthesis of 25 (OH)D
Vitamin D-dependent rickets type 2A (VDDR2A; OMIM#277440)	*VDR* (12q13.11)	↓	N, ↓	↑↑↑	↓	Varies	↓	N, ↓	↑↑↑	N	↑↑	Impaired signaling of the VDR
Vitamin D-dependent rickets type 2B (VDDR2B; OMIM#264700)	HNRNPC	↓	N, ↓	↑↑↑	↓	Varies	↓	N	↑↑↑	N	↑↑	Impaired signaling of the VDR
Vitamin D-dependent rickets type 3 (VDDR3; OMIM# pending)	CYP3A4	↓	↓	↑↑↑	↓	Varies	↓	?	↑↑↑	↓	↓	↑ inactivation of 1,25 $(OH)_2D$
Phosphopenic rickets												
Rickets and/or osteomalacia due to dietary phosphate deficiency or impaired bioavailability												
Breastfed very low birthweight infants Use of elemental or hypoallergenic formula diet or parental nutrition Excessive use of phosphate binders Gastrointestinal surgery or disorders	NA	N, ↑	↓	↑, ↑↑	?	↓	N^b	N, ↓	N	N	N, ↑	Phosphate deficiency
Rickets and/or osteomalacia with renal tubular phosphate wasting due to elevated FGF23 levels and/or signaling												
X-linked hypophosphatemia (XLH; OMIM#307800)	*PHEX* (Xp22.1)	N	↓	↑, ↑↑	↓	↑	↓	↑, N	N, $\uparrow^c$	N	N^d	↑ FGF23 expression in bone and impaired FGF23 cleavage
Autosomal-dominant hypophosphatemic rickets (ADHR; OMIM#193100)	*FGF23* (12p13.3)	N	↓	↑, ↑↑	↓	↑	↓	↑, N	N, $\uparrow^c$	N	N^d	FGF23 protein resistant to degradation

(continued)

Table 1 (continued)

Disorder (abbreviation; OMIM#)	Gene (location)	Ca	P	ALP	U_{Ca}	U_{P}	TmP/GFR	FGF23	PTH	25 (OH) D[a]	1,25 $(OH)_2D$	Pathogenesis
Autosomal-recessive hypophosphatemic rickets 1 (ARHR1; OMIM#241520)	*DMP1* (4q22.1)	N	↓	↑, ↑↑	↓	↑	↓	↑, N	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone
Autosomal-recessive hypophosphatemic rickets 2 (ARHR2; OMIM#613312)	ENPP1 (6q23.2)	N	↓	↑, ↑↑	↓	↑	↓	↑, N	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone
Raine syndrome associated (ARHR3; OMIM#259775)	*FAM20C* (7q22.3)	N	↓	↑, ↑↑	?	↑	↓	↑, N	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone
Fibrous dysplasia (FD; OMIM#174800)	*GNAS* (20q13.3)	N, ↓	↓	↑, ↑↑	↓	↑	↓	N, ↑	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone
Tumor-induced osteomalacia (TIO)	NA	N, ↓	↓	↑, ↑↑	↓	↑	↓	N, ↑	N, ↑[c]	N	N[d]	↑ FGF23 express-ion in tumoral cells
Cutaneous skeletal hypophosphatemia syndrome (SFM; OMIM#163200)	*RAS* (1p13.2)	N, ↓	↓	↑, ↑↑	↓	↑	↓	N, ↑	N, ↑[c]	N	N[d]	?
Osteoglophonic dysplasia (OGD) (OMIM#166250)	*FGFR1* (8p11.23)	N	↓	↑, N	N	↑	↓	N	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone
Hypophosphatemic rickets and hyperparathyroidism (OMIM#612089)	*KLOTHO* (13q13.1)	N	↓	↑, ↑↑	↓	↑	↓	↑	↑↑	N	N[d]	Unknown; translocation of the KLOTHO promoter
Rickets and/or osteomalacia due to primary renal tubular phosphate wasting												
Hereditary hypophosphatemic rickets with hypercalciuria (HHRH; OMIM#241530)	*SLC34A3* (9q34.3)	N	↓	↑(↑↑)	N, ↑	↑	↓	↓	Low N, ↓	N	↑↑	Loss of function of NaPi2c in the proximal tubule

X-linked recessive hypophosphatemic rickets (OMIM#300554)	*CLCN5* (Xp11.23)	N	↓	↑(↑↑)	N, ↑	↑	↓	Varies	Varies	N	↑	Loss of function of CLCN5 in the proximal tubule
Hypophosphatemia and nephrocalcinosis (NPHLOP1; OMIM#612286) Fanconi Reno-tubular syndrome 2 (FRTS2; OMIM#613388)	*SLC34A1* (5q35.3)	N	↓	↑(↑↑)	↑	↑	↓	↓	Varies	N	↑	Loss of function of NaPi2a in the proximal tubule
Cystinosis (OMIM#219800) and other hereditary forms of Fanconi syndrome)	*CTNS* (17p13.2)	N, ↓	↓	↑(↑↑)	N, ↑	↑	N, ↓	N, ↑[e]	N, ↑[e]	N	N[d]	Cysteine accumulation in the proximal tubule
Iatrogenic proximal tubulopathy	NA	N	↓	↑(↑↑)	Varies	↑	↓	↓	Varies	N	↑	Drug toxicity

N = normal; ↑ = elevated; ↑↑ or ↑↑↑ = very elevated; ↑ (↑↑) = may range widely: Ca, serum levels of calcium; P, serum levels of phosphate; ALP alkaline phosphatase; U_{Ca}, urinary calcium excretion; TmP/GFR = maximum rate of renal tubular reabsorption of phosphate normalized to the glomerular filtration rate; FGF23 = fibroblast growth factor 23; PTH = parathyroid hormone; $1,25(OH)_2D$ = 1,25-dihydroxyvitamin; 25(OH)D = cholecalciferol; NA = not applicable; [a] = cave: prevalence of vitamin D deficiency was reported to be up to 50% in healthy children; [b] = normal after restoration of P, but falsely reduced before restoration; [c] = PTH may be moderately elevated; [d] = decreased relative to the serum phosphate concentration; [e] = depending on the stage of chronic kidney disease.

Adapted from Haffner et al. (Haffner D, Emma F, Eastwood DM, Duplan MB, Bacchetta J, Schnabel D, et al. Clinical practice recommendations for the diagnosis and management of X-linked hypophosphatemia. Nat Rev. Nephrol. 8 mai 2019)

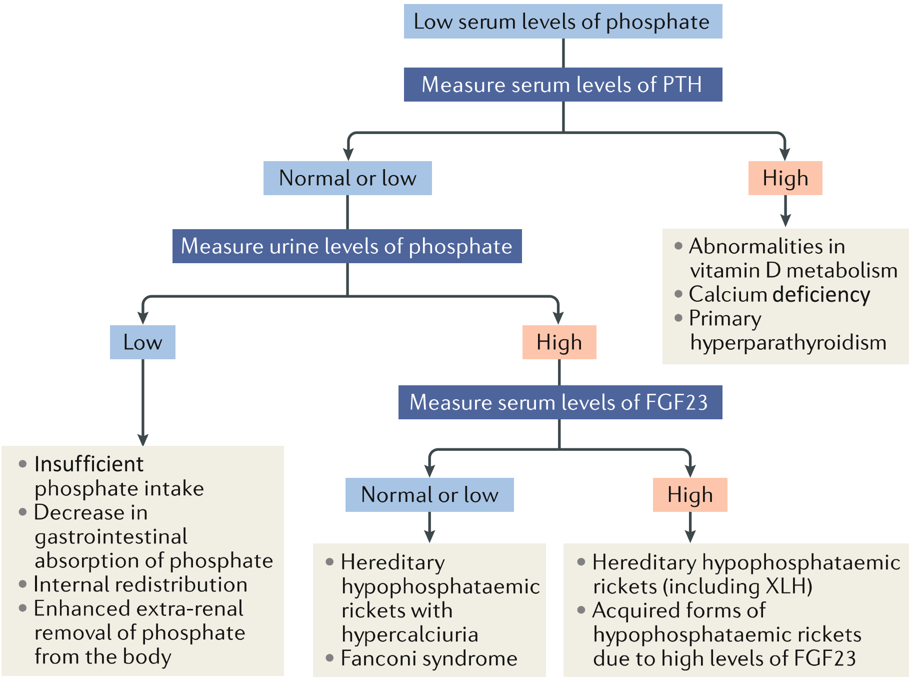

Low serum levels of phosphate
Measure serum levels of PTH
Normal or low
High
Measure urine levels of phosphate
• Abnormalities in vitamin D metabolism
• Calcium deficiency
• Primary hyperparathyroidism
Low
High
Measure serum levels of FGF23
• Insufficient phosphate intake
• Decrease in gastrointestinal absorption of phosphate
• Internal redistribution
• Enhanced extra-renal removal of phosphate from the body
Normal or low
High
• Hereditary hypophosphataemic rickets with hypercalciuria
• Fanconi syndrome
• Hereditary hypophosphataemic rickets (including XLH)
• Acquired forms of hypophosphataemic rickets due to high levels of FGF23

Fig. 7 Algorithm for the evaluation of the child with rickets presenting with hypophosphatemia. The differential diagnoses are based on the mechanisms leading to hypophosphatemia, namely high parathyroid hormone (PTH) activity, inadequate phosphate absorption from the gut, or renal phosphate wasting. The latter may be due to either primary tubular defects or high levels of circulating FGF23. Further details of individual entities can be found in Table 1; FGF-23, fibroblast growth factor 23 (Used with permission of Nature/Springer from Haffner et al. [18]

calcium, phosphate, creatinine, glucose, amino-acids, and beta2 microglobulin as well as calculation of TmP/GFR and TRP [18]. Reference values for serum and urine biomarkers used for assessment of renal hypophosphatemia are given in Table 2. Extended molecular genetic analysis may be required to establish diagnosis in unclear cases.

Hypophosphatemic Disorders with Increased FGF23 Activity

X-Linked Hypophosphatemia (*PHEX* Mutation)

XLH (OMIM #307800) is a dominant disorder with a prevalence of approximately 2/100,000 children. XLH is X-linked dominantly inherited, as it is caused by mutations in *PHEX*, located on chromosome Xp22 in ~90% of familial cases and ~ 70% of sporadic cases displaying a typical phenotype [8, 9]. The altered function of *PHEX* causes both the mineralization defect and the renal phenotypic abnormalities of XLH.

Clinical manifestations of XLH occur most often around the age of walking, despite an adequate vitamin D supplementation. In children, the primary clinical symptoms are rickets, including bone deformities, and impaired growth, dental abscesses, and craniosynostosis [18, 19, 22]. In the absence of diagnosis and/or treatment, patients may show extreme short stature with an adult height below −2 SD [26]. Tooth eruption is often delayed, but, when present, teeth display a normal enamel. The impaired mineralization of dentin is the cause of dental abscesses and early decay of lacteal and permanent teeth [27]. Many patients may have partial synostosis of the sagittal sutures leading to a dolichocephalic shape of the head. Type 1 Chiari malformation and syringomyelia are common complication of XLH mostly asymptomatic, and rarely featuring headaches and neck pain (Fig. 3) [19].

Table 2 Reference values for serum and urine biomarkers used for assessment of renal hypophosphatemia

Age- and/or sex-specific values												Age-independent specific values		
	iCa[a] mmol/L	Ca[a] mg/dL	P[a] mg/dL		TmP/GFR[b] mg/dl		TRP[b] (%)		ALPc U/L		$U_{Ca/Crea}$[a] (mol/mol)	Intact PTH[a] pg/mL	25(OH) D[a] ng/mL	1,25 (OH)2D[d] ng/L
0–5 m	1.22–1.40	8.7–11.3	5.2–8.4	**Birth**	3.6–8.6	**0–1 y**	85–98	**0–15 days**	90–273	0-1 **y.**	< 2.2	35–70	>30	40–100
6–12 m	1.20–1.40	8.7–11.0	5.0–7.8	**3 m**	3.7–8.25	**1 to < 2y**	85–98	**15–30 days**	134–518	**1 to < 3y**	< 1.4			
1–5 y	1.22–1.32	9.4–10.8	4.5–6.5	**6 m**	2.9–6.5	**2 to < 10 y**	F: 82–99 M: 85–98	**1 to < 10 y**	156–369	**3 to < 5y**	< 1.1			
6–12 y	1.15–1.32	9.4–10.3	3.6–5.8	**2–15 y**	2.9–6.5	**10– to < 16y**	F: 79–98 M: 83–97	**10 to < 13 y**	141–460	**5 to < 7y**	< 0.8			
13–20 y	1.21–1.30	8.8–10.2	2.3–4.5			**16 to < 18y**	F: 82–90 M: 82–90	**13 to < 15 y**	F: 62–280 M:127–517	**7 to < 18y**	< 0.7			

(continued)

Table 2 (continued)

Age- and/or sex-specific values												Age-independent specific values		
	iCa[a] mmol/L	Ca[a] mg/dL	P[a] mg/dL		TmP/ GFR[b] mg/dl		TRP[b] (%)		ALPc U/L		$U_{Ca/Crea}$[a] (mol/mol)	Intact PTH[a] pg/mL	25(OH) D[a] ng/mL	1,25 (OH)2D[d] ng/L
								15 to < 17 y	F: 54–128 M: 89–365					
								17 to < 19 y	F: 48–95 M: 59–164					

iCa Ionized calcium, *Ca* Calcium, *P* Phosphorus, *ALP* Alkaline phosphatase, *U* urine, *Crea* Creatinine, *PTH* Parathyroid hormone, *25(OH)D* 25-hydroxyvitamin D, *m* months, *y* years, *M* males, *F* females

Conversion factor for calcium and ionized calcium: mg/dL × 0.25 = mmol/L

Conversion factor for phosphate: mg/dL × 0.323 = mmol/L

Conversion factor for intact PTH: pg/mL x 0.11 = pmol/L

Conversion factor for 25(OH)D: ng/mL x 2.5 = nmol/L

Conversion factor for 1,25(OH)2D: ng/L x 2.5 = pmol/L

NOTE. Metric Units x Conversion Factor = SI Units

TRP is calculated by entering the fasting urine and plasma concentrations, in the same concentration units, into the following equation TRP = 1- ((Up/Pp) x (Pcr/Ucr))

If the TRP is 86% or less, then TmP/GFR = TRP X Pp

If the TRP is greater than 86%, then TmP/GFR = 0.3 x TRP/ (1-(0.8 x TRP)) x Pp

Reference: Barth JH, Jones RG, Payne RB. Calculation of renal tubular reabsorption of phosphate: the algorithm performs better than the nomogram. Ann Clin Biochem 2000; 37: 79–81

Online calculator for TmP/GFR is available at: http://www.baspath.co.uk/calculations/renal_tubular_reabsorption_of_ph.htm

References for table: [a]KDOQI Work Group. KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update. Executive Summary. Am J Kid Dis 2009 Mar;53(3 suppl 2):S11-S104 PMID: **19231749.** https://doi.org/10.1053/j.ajkd.2008.11.017. [b]Payne RB. Renal tubular reabsorption of phosphate (TmP/GFR): indications and interpretation. Ann Clin Biochem. 1998;35:201–6. Bistarakis L, Voskaki I, Lambadaridis J et al. Renal handling of phosphate in the first six months of life. *Arch Dis Child* 1986; **61:** 677–681. [c]Adeli K, Higgins V, Trajcevski K et al. The Canadian laboratory initiative on pediatric reference intervals: a CALIPER white paper. Crit Rev. Clin Lab Sci 2017; 54: 358–41. [d]Wildermuth S, Dittrich K, Schmidt-Gayk H, Zahn I. O'Riordan JL. Scintillation proximity assay for calcitriol in serum without high pressure liquid chromatography. Clin Chim Acta 1993 Oct 29;220(1):61–70. https://doi.org/10.1016/0009-8981(93)90006-p.

XLH is characterized by elevated ALP, low serum phosphate (adjusted to age, see Table 1), phosphate wasting, and elevated levels of circulating FGF23. Normal FGF23 levels do not exclude XLH, since FGF23 levels depend on other factors, e.g., phosphate intake [28]. Average intact FGF23 levels in untreated Japanese pediatric XLH patients amounted to 84.5 ± 27.4 pg/ml (lowest, 38 pg/ml). The authors proposed a cutoff level to diagnose XLH of 30 pg/ml [29]. However, FGF23 values considerably differ depending on the assay used as well as other factors (vide supra).

On radiographs, the metaphyseal signs are those of common rickets. There are no signs of bone resorption, and usually, no radiolucency. As a consequence, the Thacher score of patients with hypophosphatemic rickets is usually graded below 3 on a scale of 10 [23].

The diagnosis of XLH is based on clinical, radiological, and biochemical findings. The x-linked inheritance strongly argues for the diagnosis. FGF23 is not available everywhere and is not fully mandatory for the diagnosis. Mutational analysis of the *PHEX* gene may also contribute to distinguish XLH from other forms of rickets.

ADHR (*FGF23* Mutation)

Autosomal-dominant hypophosphatemic rickets (ADHR) (OMIM#193100) is a rare disorder that was first described by Bianchine et al. in 1971 [30]. ADHR and XLH have marked clinical similarities but differ in their modes of inheritance. ADHR is caused by activating mutations of the *FGF23* gene. They result in a mutated protein resistant to cleavage by proteolytic activity, which in turn leads to elevated, circulating FGF23 levels [31]. The consequences of elevated FGF23 levels are renal phosphate wasting, hypophosphatemia, and inappropriately low levels of 1,25(OH)$_2$D. Important to note, the penetrance of ADHR is not complete, resulting in a highly variable phenotype and, consequently, variable symptomatology and biological findings. In contrast to XLH, patients suffering from ADHR usually become symptomatic after early childhood, even in in adolescence or even during adulthood. During childhood, the clinical symptoms are similar to that observed in XLH, i.e., rickets with bone deformities, disproportionately short stature, and dental abnormalities (abscesses) [32]. If patients become symptomatic after puberty, complications due to osteomalacia, e.g., weakness, fatigue, bone pain, and pseudofractures, are the major symptoms. XLHR adults present with symptoms similar to those with tumor-induced osteomalacia (TIO), as discussed below. Interestingly, some children show improvement of phosphate wasting during puberty [33]. Studies in ADHR mice and humans suggest that iron status is an important regulator of FGF23-metabolic pathways [33–35]. The onset of ADHR is probably the product of gene-environment interactions. In ADHR patients' serum, iron was negatively associated with both C-terminal FGF23 and intact FGF23 plasma levels. Similarly, a negative association between serum iron and C-terminal FGF23 was observed in healthy subjects, suggesting increased expression and cleavage of FGF23 in the setting of a low iron status. In line with this observation, a recent study in iron-deficient adult ADHR patients demonstrated normalization of both, serum FGF23 and phosphate levels by oral iron repletion [36]. Therefore, the standard approach to ADHR patients should also include recognition and management of iron deficiency.

ARHR (1, *DMP1* Mutation; 2, *ENPP1* Mutation)

The finding of hypophosphatemic rickets in consanguineous kindreds suggested an autosomal recessive form of hypophosphatemia (ARHR) [37]. The clinical presentation is similar to those of XLH patients including elevated FGF23 serum levels, renal phosphate wasting, and inappropriately normal levels of 1,25(OH)$_2$D. One difference is, however, that patients with ARHR show a relatively high bone density of the vertebral bodies on X-rays. ARHR is either due to mutations in the *DMP1* gene encoding for dentin matrix acidic phosphoprotein 1 (ARHR1) or to mutations in the *ENPP1* gene encoding for ectonucleotide pyrophosphatase/phosphodiesterase 1 (ARHR2) [38–41]. DMP1 is a member of the short integrin-binding ligand interacting N-linked glycoprotein (SIBLING) family of skeletal matrix proteins and is highly expressed in mineralized tissues, e.g., in osteoblasts and osteocytes. It is an important regulator of development of bone, cartilage, and teeth. How mutations in *DMP1* result in elevated FGF23 serum levels in ADHR1 patients is largely elusive. Levi-Litan et al. identified an inactivating mutation in the *ENPP1* gene (later named ARHR2) that caused ARHR in a Bedouin family [41]. ENPP1 is a cell surface protein that catalyzes phosphoester cleavage of adenosine triphosphate, generating the mineralization inhibitor pyrophosphate. Mutations in *ENPP1* were initially reported in patients with infantile arterial calcifications [42]. There is a high phenotypic heterogenity, and the clinical presentations may markedly differ in patients displaying the same biallelic *ENPP1* pathogenic variants including enthesopathy and primary hyperparathyroidism [43]. How inactivating *ENPP1* gene mutations result in increased FGF23 synthesis from bone is unknown.

Raine Syndrome Associated *FAM20C* Mutation

Raine syndrome (OMIM#611061) is an ultrarare disease caused by biallelic loss of function mutations in FAM20C. FAM20C is a key-regulator of osteoblasts/osteocytes; it also promotes FGF23 cleavage. The disease is characterized by generalized osteosclerosis, craniofacial anomalies including hypoplastic nose, midface hypoplasia, exophthalmos, intracranial calcifications, sensorineural hearing loss, developmental delay, epilepsy, large fontanelle, and amelogenesis imperfecta. Patients may display from a mild phenotype with hypophosphatemia and renal phosphate wasting due to elevated FGF23, to severe and lethal dysmorphic features [44].

Tumor-Induced Osteomalacia and Tumor-Induced Rickets (TIO)

Tumor-induced osteomalacia (TIO) is a rare acquired cause of hypophosphatemia and phosphate wasting. It is caused by the excessive production of phosphaturic factors including FGF23 by benign tumors, slowly growing, characterized as polymorphous neoplasms made of mixed connective tissue. These tumors are often small and difficult to localize through conventional imaging techniques. TIO occurs in adults, rarely in adolescents or children. There is no familial or personal history of rickets, and patients present with fractures, bone pain, fatigue, muscular insufficiency, hypocalcemia, hypophosphatemia, and high levels of FGF23. In comparison with XLH, mean phosphate and FGF23 levels are dramatically lower and higher in patients with TIO [36, 45, 46]. Normalization of phosphate homeostasis and osteomalacia occurs after the surgical removal of tumors; as surgery is sometime not possible, therapies aiming at lowering FGF23 levels and actions allow an improvement in phosphate level, renal tubular reabsorption of phosphate, muscular function, and quality of life of affected patients [47].

Hypophosphatemic Rickets and Hyperparathyroidism

In one patient with hypophosphatemic rickets, there were skeletal abnormalities and hyperparathyroidism due to parathyroid hyperplasia a de novo translocation with a breakpoint adjacent to α-Klotho, a factor known to regulate FGF signaling. In this patient, plasma α-Klotho and FGF23 levels were markedly elevated [48].

Fibrous Dysplasia (FD), McCune-Albright Syndrome (MAS), and Epidermal Neavi

Rickets occurs sometimes in patients with fibrous dysplasia (FD) due to activating mutations in GNAS observed in McCune-Albright syndrome. Briefly, in FD tissue, increased production and cleavage of FGF23 occurs. The degree of FGF23 overproduction correlates with the volume of FD lesions, i.e., disease severity and skeletal burden. Hypophosphatemia is usually mild and associated with phosphate wasting. However, the clinical consequences of FGF23-mediated hypophosphatemia may include earlier onset of bone features, increased risk of fractures, and FD-related bone pain. The evolution is peculiar as it may resolve as patients become older, likely reflecting the decline in the number of mutated stromal cells and the tendency of FD histologic features to improve over time [49].

Rarely, elevated serum FGF23 levels are found in association with mosaic cutaneous disorders that affect large proportions of the skin and appear in patterns corresponding to the migration of ectodermal progenitors. These disorders are named Epidermal nevi and congenital melanocytic nevi (EN and CMN) as well as Schimmelpenning–Feuerstein–Mims syndrome. The wide distribution of involved tissues and the appearance of multiple segmental skin and bone lesions are compatible with early embryonic somatic mutations. Indeed, in affected patients, exome sequencing of blood, dysplastic bone, and affected skin tissue identified somatic activating mutations of *HRAS* or *NRAS*. Elevated serum FGF23 is therefore the consequence of the somatic activating RAS mutation in bone [50].

Hypophosphatemic Disorders with Normal or Suppressed FGF23 Activity

HHRH (*SLC34A3* Mutation)

This important differential diagnosis to XLH was first described by 1985 Tieder et al. in 1985 reporting on an unusual case of hypophosphatemic rickets in a consanguineous Bedouin tribe [51]. In contrast to XLH, in HHRH (OMIM#241530) hypophosphatemia and phosphate wasting are associated with elevated 1,25(OH)$_2$D serum levels resulting in hypercalciuria and suppressed PTH plasma concentrations. The disorder was later shown to be caused by mutations in the *SLC34A3* gene encoding for the NaPi-IIc renal phosphate cotransporter in the proximal tubule, and many more patients were identified with different ethnicities from all over the world [52]. All so far reported mutations are loss-of-function mutations and, most likely, result in diminished renal phosphate absorption through decreasing the apical membrane expression of NaPi-IIc or the uncoupling of sodium-phosphate cotransport in the proximal tubule [52]. Thus, in contrast to XLH and ADHR, hypophosphatemia is not due to enhanced FGF23 serum levels in HHRH. The physiological consequence of the body facing hypophosphatemia is to increase 1,25(OH)$_2$D levels in order to enhance intestinal calcium absorption and suppress PTH levels. Unfortunately, this results in hypercalciuria promoting the development of nephrocalcinosis and nephrolithiasis. Milder forms may be underdiagnosed, and therefore, careful evaluation of urinary calcium excretion before and during medical treatment is strongly recommended in all patients with hypophosphatemic rickets, especially in those without a proven underlying genetic cause [53].

Hypophosphatemia and Nephrocalcinosis (*SLC34A1* Mutation, OMIM#612286)

The *SLC34A1* gene is located on chromosome 5q34 encoding for the NaPi-IIa renal phosphate cotransporter in the proximal tubule. *SLC34A1* mutations may result in a wide clinical phenotype including infantile hypercalcemia, kidney stones, and rickets, all of which are associated with reduced serum phosphate levels [54, 55].

Osteoglophonic Dysplasia (OMIM#166250)

Patients with mutations of FGFR1 (Fibroblast growth factor receptor 1) causing ligand-independent activation of the receptor have been found in osteoglophonic dysplasia. OGD is a rare disease characterized by craniosynostosis, facial dysmorphism, failure of tooth eruption, and variable short stature; some patients, but not all, develop hypophosphatemic rickets secondary to upregulated bony FGF23 secretion hence elevated FGF23 levels [56, 57].

Nephrolithiasis and Osteoporosis Associated with Hypophosphatemia

Two different heterozygous mutations (A48P and V147M) in *NPT2a*, a gene encoding a sodium-dependent phosphate transporter, were reported in patients presenting with urolithiasis or osteoporosis and persistent hypophosphatemia due to decreased tubular phosphate reabsorption [54, 58].

Fanconi Syndrome

De Toni Debré Fanconi syndrome is characterized by generalized proximal tubular dysfunction with impaired ability to absorb water, phosphate, glucose, urate, amino acids, and low-molecular weight proteins. Other common features include increased excretion of sodium, potassium, calcium, and bicarbonate. Clinical consequences result mainly from hypophosphatemia and metabolic acidosis, i.e., rickets and osteomalacia. The syndrome may be due to both genetic defects and acquired disorders with nephropathic cystinosis being the most common cause (see chap. XX). In the latter, FGF23 levels are inadequately low despite progressive CKD due to persistent hypophosphatemia [24].

Iatrogenic Phosphate Wasting

Low phosphate, decreased phosphate tubular reabsorption rate, and TmP/GFR have been firmly associated with exposition to certain chemotherapies such as cisplatin and ifosfamide [59]. This rarely results in clinical consequences; despite a significant reduction in tubular threshold for phosphate in ~25% of the children, hypophosphatemia is present only in ~1% [60]. Other drugs may also impair the renal tubular function inducing tubulopathy of variable severity; Fanconi syndrome and phosphate wasting have been described in patients receiving tenofovir or other protease inhibitors [61].

Treatment of XLH

The objective of the treatment is to heal rickets, restore the lower limbs shape, and improve growth, bone and teeth mineralization, and muscular function. A multidisciplinary approach is therefore mandatory to prevent the development of endocrine, orthopedic, rheumatologic, and renal complications. Disease management encompasses social aspects, patient/family education and support [17, 18].

Phosphate and Active Vitamin D Metabolites (Children and Adults)

In Children

Vitamin D analogs (alfacalcidol or calcitriol) and phosphate supplements through multiple daily dosing are used to counteract the calcitriol deficiency secondary to the increased FGF23 concentrations, and to compensate for renal phosphate wasting. Doses vary from 10–80 ng/kg/day of calcitriol and 30–60 mg/kg/day of elemental phosphorus. The efficacy of treatment should be regularly assessed through the measurement of ALP, physical measures of limb deformities, evaluation of the function through 6-minute walk test or POSNA, and PODCI subscales of height and growth velocity.

The recommended starting dose of phosphate based on elemental phosphorus is 20–60 mg/kg/day (0.7–2.0 mmol/kg per day) in infants and preschool children, which should be adjusted according to the improvements of rickets, growth, and ALP and PTH levels [18]. The recommended starting dose of calcitriol and alfacalcidiol is 20–30 ng/kg body weight and 30–50 ng/kg body weight daily, respectively. Alternatively, treatment may be started empirically at 0,5 μg daily of calcitriol or 1 μg daily of alfacalcidiol in patients aged above 12 months and adjusted on the basis of clinical and biochemical response. In addition, supplementation with native vitamin D is recommended in case of vitamin D deficiency.

This therapy has demonstrated its efficacy as it leads to a decrease in ALP to the upper limit of normal in ~1 year, an improvement in bone deformity, bone pain and gait in 30–60% of patients, an improvement in growth velocity of ~1 standard deviation, a significant improvement of dentin mineralization, and therefore a decrease of teeth abscesses and oral complications in affected children. It was clearly shown that early treatment initiation and longer duration of treatment are associated with better outcomes.

Phosphate and FGF23 levels, TmP/GFR or tubular reabsorption rate of phosphate, do not reflect treatment efficacy and should therefore not be used to guide treatment with phosphate and active vitamin D.

Long-term treatment with vitamin D analogs and phosphate supplements is associated with an increased incidence of nephrocalcinosis and/or urolithiasis, elevated levels of PTH, and sometimes secondary hyperparathyroidism. Therefore, safety should be considered at each evaluation by measuring serum levels of calcium and PTH, urinary calcium excretion, and renal ultrasound [18]. Other complications may occur during the course of treatment including insufficient improvement of bone shape leading to iterative corrective surgeries of lower limbs, impaired muscle function, craniosynostosis, and neurological features, e.g., Chiari 1 and syringomyelia, hearing problems, enthesopathy, chronic pain, and asthenia [6, 19, 62].

In Adults

Data show that, in adults, treatment may prevent or limit the consequences of chronic phosphate wasting such as osteomalacia-related fractures and pseudofractures, muscle impairment as well as dental problems.

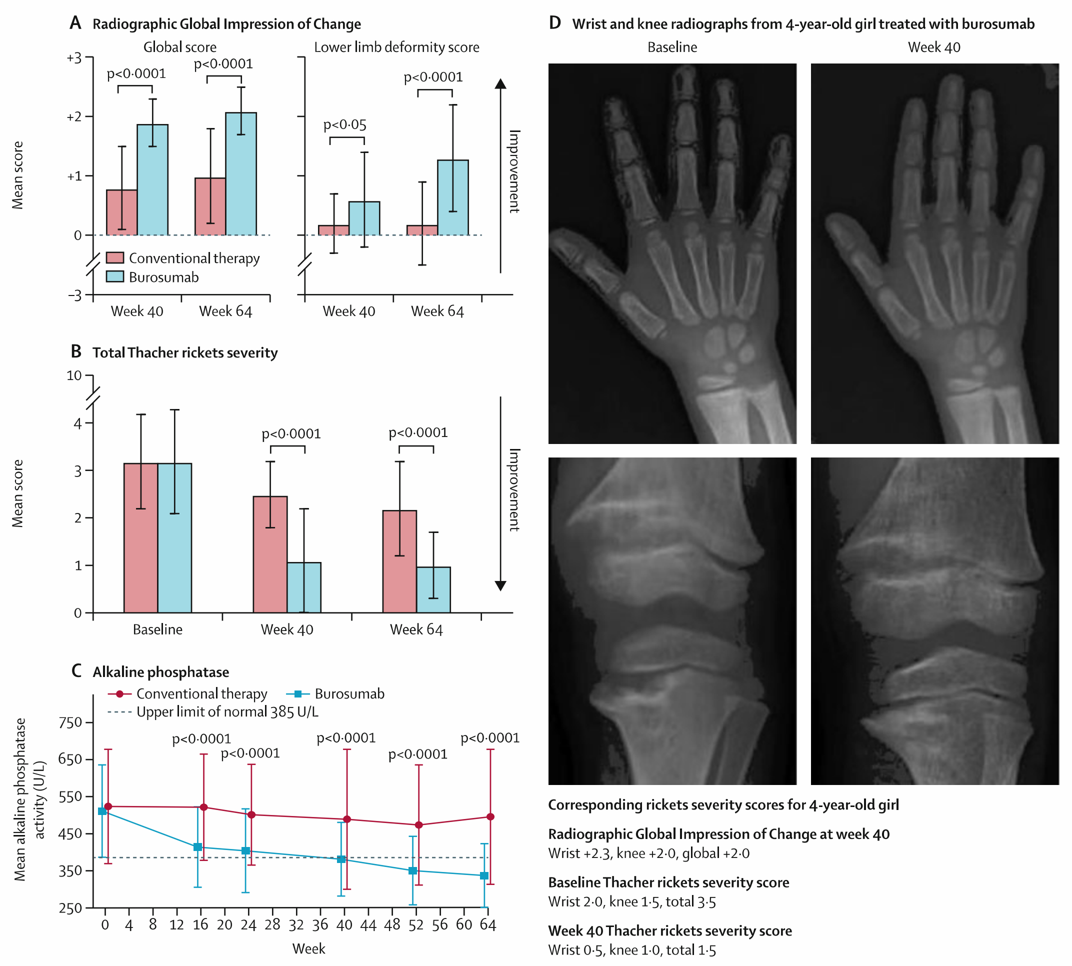
Oral phosphate salts and active vitamin D analogs are prescribed in symptomatic adults to reduce bone pain and improve osteomalacia, muscle strength, and physical function. Spontaneous insufficiency fractures are more likely to heal faster with therapy. It is also recommended before and after surgical orthopedic or dental interventions to promote bone mineralization. The treatment does not prevent the occurrence of enthesopathy and does not modify osteoarthrosis-related pain.

In asymptomatic patients, treatment to prevent long-term consequences of chronic hypophosphatemia should be weighed with the potential side effects such as hyperparathyroidism and nephrocalcinosis. However, it seems appropriate during pregnancy, lactation, and menopause, conditions associated with an increased calcium and phosphate demands.

Oral phosphate salts are given usually at 750–1600 mg/day (based on elemental phosphorus) in three to four doses; they should be avoided in patients with increased levels of PTH. In addition, active vitamin D analogs are given to improve bone mineralization, including unalfacalcidiol 0.75–1.5 μg once daily or calcitriol 0.75–1.0 μg twice daily. 25OHD deficiency should be corrected [18, 63].

Burosumab (Children and Adults)

Since 2018, the efficacy and safety of burosumab treatment was investigated in three clinical trials in children with XLH [64–66]. Two open-label uncontrolled trials testing burosumab in a total of 65 children aged 1–12 years with severe XLH demonstrated that in the short term (12–16 months) burosumab resulted in the following outcomes: a significant increase in TmP/GFR and consequently raised serum phosphate levels into the lower end of the normal range, with increased (normalized) 1,25(OH)$_2$ vitamin D levels; a significant reduction in the severity of rickets (as measured by the Rickets Severity Score (RSS) and the Radiographic Global Impression of Change (RGI-C)); a significant improvement in physical ability (as measured by walking distance in the Six Minute Walk Test); and a significant reduction in patient-reported pain and functional disability (as measured by the use of the Pediatric Orthopedic Society of North America Outcomes Data Collection Instrument) [64, 65]. Recently, a direct comparison between burosumab and conventional treatment was investigated in an open-label phase 3 trial including 61 children aged 1–12 and lasted 64 weeks [66–71]. The primary outcome analysis at 40 weeks showed that children treated with burosumab had significantly greater improvement in RGI-C and RSS than did patients in the conventional therapy group (Fig. 8 and 6**)**. Likewise, biochemical parameters such as ALP and serum phosphate levels were also rapidly normalized during burosumab treatment. The most common adverse reactions observed with burosumab were injection site reactions, headache and pain in the extremities. Two-weekly doses were superior to four-weekly doses with respect to normalization of serum levels of phosphate and radiological improvement of rickets. Conventional treatment should be stopped at least 1 week before the start of burosumab therapy for washout and to prove that fasting serum phosphate levels are below the normal reference for age. Supplementation with native vitamin D may be continued in patients under burosumab treatment.



Corresponding rickets severity scores for 4-year-old girl

Radiographic Global Impression of Change at week 40
Wrist +2.3, knee +2·0, global +2·0

Baseline Thacher rickets severity score
Wrist 2·0, knee 1·5, total 3·5

Week 40 Thacher rickets severity score
Wrist 0·5, knee 1·0, total 1·5

Fig. 8 Improvements in rickets severity in children on burosumab versus conventional therapy with phosphate and active vitamin D metabolites. Data in panels A, B, and C are reported as mean (SD). p values are based on the comparison between treatment groups in the least squares mean change from baseline, using the ANCOVA model for the week 40 Radiographic Global Impression of Change global score and week 40 Thacher rickets severity score assessments, and the generalized estimating equation model for alkaline phosphatase assessments, lower limb deformity assessments, and week 64 rickets assessments. The upper limit of normal for alkaline phosphatase varies by age and sex: girls aged 1–4 years 317 U/L, 4–7 years 297 U/L, 7–10 years 325 U/L, and 10–15 years 300 U/L; boys aged 1–4 years 383 U/L, 4–7 years 345 U/L, 7–10 years 309 U/L, and 10–15 years 385 U/L. These ranges were provided by Covance laboratories. Radiographs in panel D show improvement in rickets with burosumab in a 4-year-old girl who previously received conventional therapy for approximately 26 months (Used with permission of Elsevier from Imel et al. [66]

Two open-label, uncontrolled trials and one randomized, double blind, placebo-controlled study (including a total of 176 patients) have investigated burosumab in adult XLH patients, and the majority presented with skeletal pain associated with XLH and/or osteomalacia [67–71]. Four-weekly doses of burosumab given over periods of 4–12 months were associated with the following outcomes: significantly increased TmP/GFR and consequently raised serum levels of phosphate into the lower normal range and increased (normalized) 1,25 $(OH)_2$ vitamin D levels; healed osteomalacia, and accelerated healing of active fractures and pseudofractures; and significantly reduced stiffness (as measured by the Western Ontario and the McMaster Universities Osteoarthitis Index (WOMAC) stiffness subscale). However, reductions in WOMAC physical function subscale and Brief Pain Inventory did not achieve statistical significance when compared to placebo. Notably, all studies to date in adults with XLH took place in moderately to severely affected patients, making extrapolation to real world patients who may present with a milder phenotype difficult [63, 72]. The reported adverse events were similar to that observed in the pediatric trials (that is, injection site reactions, headache and pain in the extremities).

Based on these studies, the European Medicines Agency (EMA) approved burosumab in the European Union for the treatment of XLH with radiographic evidence of bone disease in children $\geq$1 year and in adolescents with a growing skeleton. The FDA granted approval of burosumab to treat adults and children $\geq$1 year with XLH. Recently, EMA approved burosumab for children $\geq$1 year and adults with XLH as well. However, several open issues need to be addressed. First, in all trials performed to date, the dose of burosumab was adjusted to achieve serum phosphorus level at the low end of the normal range. The optimal therapeutic strategy, including dosing frequency and target phosphate levels, may differ in patients who have achieved a satisfactory phenotypic improvement. Second, in view of the high cost of burosumab and lack of long-term efficacy and safety data, there is a need to better define patients who will benefit the most. Because the pediatric trials were restricted to patients aged between 1 and 12 years of age, the efficacy and safety of burosumab in infants and during the pubertal spurt needs to be further evaluated. The short-term improvement of growth was modest in children treated with burosumab, and it is unknown whether treated children will experience sustained catch-up growth and achieve normal adult height. Finally, the impact of burosumab on other severe complications of XHL, such as dental abscesses and sensorineural hearing loss, needs to be addressed in future studies [18, 72].

Thus, conclusive recommendations on the use of burosumab are premature. However, given the severity of the disease in some patients and the encouraging results that have prompted the EMA and FDA to grant authorization, preliminary recommendations were recently established by an European expert panel which are outlined below.

Burosumab in Children with XLH

If available, burosumab treatment should be considered in children with XLH $\geq$ 1 year and in adolescents with growing skeletons in the following situations: radiographic evidence of overt bone disease; disease that is refractory to conventional therapy; complications related to conventional therapy; or patient's inability to adhere to conventional therapy, presumed that adequate monitoring is feasible [18]. Burosumab should be started at doses of 0.4 mg/kg body weight or 0.8 mg/kg body weight, given every 2 weeks subcutaneously. Burosumab should be titrated in 0.4 mg/kg increments to raise fasting serum phosphate levels within the lower end of the normal reference range for age, to a maximum dosage of 2.0 mg/kg body weight (maximum dose 90 mg). The half-life of burosumab is approximately 19 days, and the peak serum concentration of burosumab occurs at 7–11 days after injection. Therefore, it is was suggested to monitor fasting serum phosphate levels during the titration period between injections, ideally 7–11 days after the last injection in order to detect hyperphosphatemia [18]. After achievement of a steady state, which can be assumed after 3 months of a stable dosage, fasting serum phosphate levels should be preferably assessed directly before injections in order to detect hypophosphatemia. In some patients, burosumab might initially improve TmP/GFR while serum level of phosphate is still below the normal range owing to the high demand for phosphate of the bone. Therefore, it was recommended analyzing TmP/GFR together with fasting serum phosphate levels as a measure of drug efficacy. Although no overt hypervitaminosis D was observed during clinical trials, serum levels of 1,25(OH)$_2$ vitamin

D usually increase under burosumab therapy. Therefore, measuring these levels every 6 months and analyzing them together with the urinary calcium excretion as safety parameters are recommended. Burosumab should be withdrawn if fasting serum phosphate level is above the upper range of normal. Burosumab can be restarted at approximately half of the previous dose when serum phosphate concentration is back within the normal range. Important to note, burosumab must not be given in conjunction with conventional treatment, when fasting phosphate levels are within the age-related normal reference range before initiation of treatment, or in the presence of severe kidney impairment.

Burosumab in Adults with XLH

If available, burosumab treatment should be considered in adults with XLH with the following features: persistent bone and/or joint pain owing to XLH and/or osteomalcia that limits daily activities; pseudofractures or osteomalacia-related fractures; or insufficient response or refractory to conventional therapy [18, 63]. It is also recommended to consider burosumab treatment if patients experience complications related to conventional therapy.

The starting dose of burosumab is 1.0 mg/kg body weight (maximum dose of 90 mg) given every 4 weeks subcutaneously. Fasting serum phosphate levels should be evaluated between injections, ideally 7–11 days after the last injection [18]. After achievement of a steady state, which can be assumed after 3 months of a stable dosage, serum levels of phosphate should be assessed during the last week before the next injection. The dose should be held if fasting serum phosphate level is above the upper limit of normal. Burosumab can be restarted at approximately half of the previous dose when serum phosphate concentration is back within the normal range. Burosumab must not be given together with conventional treatment, in patients with phosphate levels within the age-related normal reference range before initiation of treatment, or in the presence of severe renal impairment.

Adjunctive Therapies

Growth Hormone

Adult heights are reduced in up to 60% of XLH patients, and short-term burosumab treatment did not substantially improve longitudinal growth in pediatric XLH patients in clinical trials as summarized in Haffner et al. [18, 73]. Although an impairment of the growth hormone (GH) insulin-like growth factor-1 axis is not the primary cause of short stature in XLH patients, the physiological antiphosphaturic effect of GH by stimulation of both renal phosphate reabsorption and 1,25 $(OH)_2D$ synthesis is a useful adjunct to conventional treatment in improving growth in poorly growing XLH patients. Several mostly uncontrolled studies and a placebo-controlled randomized trial have documented sustained increases in standardized height during treatment periods of up to 3 years. In general, prepubertal patients responded better to GH than pubertal patients [73]. Zivicnjak et al. reported on a European multicenter randomized trial on 3-year GH treatment in severely short (< -2.5 SD score (SDS)) prepubertal XLH patients [74]. Standardized height was increased in the GH group by 1.1 SDS with no change in body disproportion, whereas no significant change in standardized height was noted in controls. In line with previous uncontrolled studies, a transient rise in TmP/GFR, and consequently of serum phosphate concentrations, was noted during the first 6 months of GH treatment but not in controls. However, long-term follow-up of the same study failed to show significant benefits on the adult height most likely due to the low number of patients being followed-up until adult height, whereas in another study mean final height was significantly higher when compared to nonrandomized controls [75, 76]. Therefore, administration of GH should be considered in XLH patients with persistent short stature despite adequate metabolic control [18]. However, the efficacy of GH treatment in patients concomitant to burosumab therapy is currently unknown.

Calcimimetics

The calcimimetic agent cinacalcet was shown to reduce PTH levels in XLH patients, resulting in increases in TmP/GFR and serum phosphate [77, 78]. The cinacalcet-induced decrease in serum PTH was accompanied by a slight decrease in serum-ionized calcium concentrations and was not associated with clinical symptoms. Similarly, Geller et al. proved the efficacy of cinacalcet in increasing serum phosphate levels in patients with TIO [79]. It was suggested that long-term adjunctive treatment of XLH patients with cinacalcet may allow for lower doses of phosphate and calcitriol and thus may minimize the risk of secondary hyperparathyroidism, hypercalcemia, hypercalciuria, and nephrocalcinosis caused by high-dose phosphate and calcitriol treatment [18]. Treatment with the calcimimetics R568 alone did not improve serum phosphate levels nor the mineralization defect in *Hyp* mice, a murine animal model of XLH supporting the concept that calcimimetics should be given together with conventional treatment [80]. However, studies on the long-term efficacy and safety of this drug in XLH patients are lacking.

Surgical Care and Dental Care

As stated above, treatment aims at improving bone and teeth mineralization.

At the end of growth, the objective is to reach a neutral axis of lower limbs with knee and ankle horizontal joints. When corrective surgery is required, the bone deformities are modified through one or multiple osteotomies associated with internal or external osteosynthesis. The risk of recurrence is high, especially when the patient is young at first surgery, even in expert hands likely due to the pathophysiology of the disorder [62].

Hemiepiphysiodesis is an alternative procedure which may correct bone deformity in young patients that is proposed as guidance for growth. Caution is needed in XLH as midterm and long-term evaluations have not been produced [18].

The occurrence of endodontic infections is dramatically reduced by the early implementation of XLH treatment with vitamin D analogs and phosphate supplements restoring oral health of supplemented children similar to that of age-matched controls [20]. Both decidual and permanent teeth can benefit from the supplementation. Treatment of endodontic infections depends on the extent of the infection, the recurrence of abscesses and the expected timing of exfoliation of the tooth. Systemic antibiotics are used in the case of an acute abscess.

The prevention of endodontic infections is actually one of the goals of conventional pharmacological management of XLH. In addition to an appropriate care of XLH, prevention is supported by local prophylactic treatments such as sealing the pits and fissures of the surface of the tooth. Early and regular dental controls are recommended to ensure an optimal dentin mineralization and formation [18, 27, 81].

Expected Outcomes

Children with XLH have normal length at birth. Growth retardation, hypophosphatemia, and rickets occur during the first year of life [82, 83]. With conventional treatment, growth and skeletal deformities generally improve. Height velocity commonly increases during the first year of treatment. Leg deformities may correct during conventional treatment obviating the need for surgery, although this is not always the case. Despite general growth improvement during treatment, correction is limited and adult height is often compromised. Median adult height in XLH patients on conventional treatment (median age at start 2.3 years) reported during the last two decades amounted to -2.3 SDS (range -2.7 to -1.2 SDS) [84, 85]. Growth outcome is significantly better if treatment is initiated early (< 1 year). In three studies, the mean standardized height after treatment periods of approx. 10 years was substantially higher in XLH patients with early compared to late treatment (-0.7 SDS versus -2.0 SDS, $p < 0.01$; -1.3 SDS versus -2.0 SDS, $p = 0.06$; Fig. 9; and -0.7 SDS versus -2.0 SDS, $p < 0.01$) [85–87]. However, even early treatment does hardly normalize skeletal development, and the main effect of early treatment was the prevention of a severe height deficit during early childhood.

Zivicnjak et al. reported on age-related stature and linear body dimensions in 76 children with XLH [88]. Despite conventional treatment, they

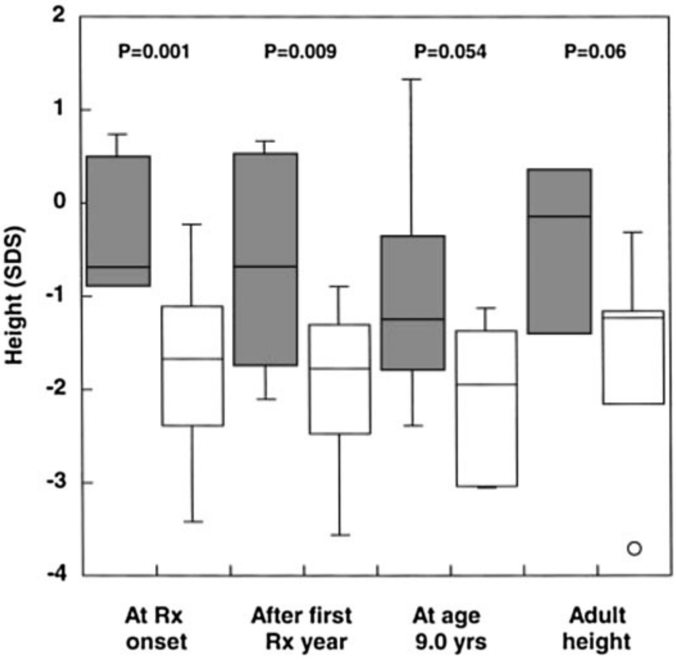


Fig. 9 Height z-scores in XLH patients started on calcitriol and phosphate treatment within the first 12 months of life 1 (■) and after the age of 12 months (□) at treatment onset, at the end of the first treatment year, at age 9 years, and at final height (adult height or predicted adult height). The bottom of each box indicates the 25th, the cross line the 50th, and the top the 75th percentile; the bottom and top lines indicate the minimum and maximum values. P values refer to the difference between groups. Rx, Treatment (Used with permission of Makitie et al. [86]

showed progressive growth failure and body disproportion during childhood, which was mainly due to diminished leg growth, whereas growth of the trunk was less affected (Fig. 10). Consequently, the sitting height index (i.e., ratio between sitting height to stature) increased with increasing age. Thus, despite medical treatment, XLH patients show uncoupled growth of legs and trunk during childhood, which in turn aggravates the preexistent disproportion between trunk and leg length. Important to note, the degree of leg bowing was only weakly associated with leg length, explaining approximately 15% of the overall variability. The finding that the lower limbs are more severely affected than the upper limbs and spine in XLH most likely reflects the fact that legs, as weight-bearing extremities, are usually exposed to higher mechanical stress loads than the upper limbs and the spine. Furthermore, growth plate activity, which in turn determines the rate of endochondral bone formation and thus bone length, is usually highest around the knees. Thus, this region is also highly prone to the development of rickets and growth impairment.

A recent North American cross-sectional study in 232 adult XLH patients all being treated with conventional therapy during childhood and adolescence revealed a high prevalence of short stature (80%), bone or joint pain/stiffness (97%), and history of pseudofractures (44%) [89]. In addition, osteophytes, enthesopathy, and spinal stenosis were reported in 46%, 27%, and 19% of patients, respectively. Similar results were reported in several European studies [90–92]. Reduced quality of life was shown in 84% of adult patients and associated with age and presence of structural lesions such as enthesopathy [90].

Treatment with burosumab results in substantial improvement of ricketic bone lesions and degree of leg deformities and nonsignificant changes in standardized height in children aged 1–12 years treated for periods of up to 16 months (vide supra). The latter may be due to rather short observation periods and/or a primary osteoblast defect in XLH. Long-term outcome data in children on burosumab treatment with respect to linear growth, dental health, and rare complications such as hearing loss are currently lacking, and multinational patient registries have been initiated to address these issues.

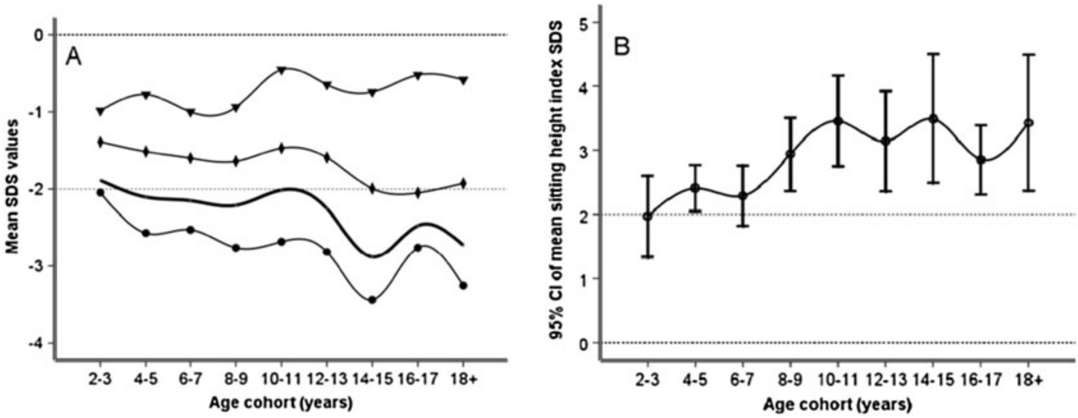


Fig. 10 Stature, sitting height, and arm and leg length (**a**) as well as the sitting height index (**b**) as a function of age in 76 children with XLH on conventional treatment. To assess age-related changes in body dimensions, all measurements were grouped according to age at the time of examination. Each age cohort comprised 8 up to 26 children with 16 up to 38 measurements. A spline function was used to fit age-related changes. Unmarked solid line stature, solid line with filled circles leg length, solid line with filled rhombuses arm length, and solid line with filled inverted triangles sitting height. The 95% CI is indicated for sitting height index [88]

Treatment of Other Forms of Hypophosphatemic Rickets

The management of patients with hypophosphatemia due to primary renal disease, i.e., tubulopathy, should be extremely cautious. Hypophosphatemia should be supplemented by multiple daily phosphate supplements using doses similar to that of XLH; large doses of cholecalciferol should be avoided, to prevent the increased production of calcitriol; in addition, 25OHD, PTH, and urinary excretion of calcium should be monitored regularly. A close follow-up of vitamin D levels – to be maintained at the lower range of normal – and of renal function is recommended in these patients [24].

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Inherited Disorders of Sodium and Potassium Handling

41

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Abstract

Bartter and Gitelman syndromes are rare, recessively inherited tubular disorders which are associated with hypokalemia and hypochloremic metabolic alkalosis due to stimulation of the renin-angiotensin-aldosterone system by a primary defect in salt (NaCl) handling in the thick ascending limb and distal convoluted tubule, respectively. These disorders differ in terms of age of onset, severity, presence of urinary concentrating defect and/or hypomagnesemia, and magnitude of urinary calcium excretion, reflecting the tubular segment of origin. Type 1 pseudohypoaldosteronism (PHA1) is a rare form of mineralocorticoid resistance characterized by neonatal kidney salt wasting, failure to thrive, and dehydration. The disease can be dominantly inherited, restricted to the kidney, or recessive, where mineralocorticoid resistance is affecting multiple organs.

Keywords

Salt wasting · RAAS activation · Hypokalemia · Metabolic alkalosis · Hypomagnesemia · Hyperkalemia · Aldosterone resistance

Bartter Syndromes

Introduction

In 1962, F. Bartter and coworkers described two African American individuals presenting a novel association of kidney salt wasting, hyperaldosteronism secondary to volume contraction, hypokalemic metabolic alkalosis, hypertrophy, and hyperplasia of the juxtaglomerular apparatus, with normal/low blood pressure (BP) [1]. These subjects also exhibited polyuria, polydipsia, muscle weakness, and growth retardation. The disorder also featured increased urinary excretion of prostaglandins, high plasma renin activity, and a resistance to the pressor effects of exogenous angiotensin II [1]. For decades, many similar cases and several phenotypic variants have been progressively identified and included in a group of hypokalemic salt-losing tubulopathies, referred to as “Bartter-like syndromes” [2]. These rare tubular disorders, which are recessively inherited, are associated with hypokalemia and hypochloremic metabolic alkalosis due to stimulation of the renin-angiotensin-aldosterone system (RAAS). However, they differ in terms of age of onset, severity, presence of urinary concentrating defect and/or hypomagnesemia, and magnitude of urinary Ca^{2+} excretion. Over the years, it became apparent that these tubulopathies primarily affect salt (NaCl) handling in distinct nephron segments, based on the similarities between clinical features and the effects of loop and thiazide diuretics on the thick ascending limb (TAL) and the distal convoluted tubule (DCT), respectively.

In the adult, differentiated nephron, the TAL reabsorbs ~25% of the filtered NaCl load. The apical electroneutral cotransporter NKCC2 mediates the uptake of Na^+, K^+, and Cl^- from the lumen into the tubular cells, driven by the electrochemical gradient established by the basolateral Na^+-K^+-ATPase. The K^+ channel ROMK recycles K^+ across the apical membrane, generating a lumen-positive voltage that facilitates the paracellular reabsorption of cations including Ca^{2+} and Mg^{2+}. Basolateral ClC-Ka and ClC-Kb Cl^- channels coupled to their regulatory beta-subunit barttin are responsible for the exit of Cl^- (Fig. 1). These transport processes are crucial for the reabsorption of NaCl in the TAL and thus the urinary concentrating ability [3]. The importance of NKCC2 in the TAL is evidenced by the effects of loop diuretics which, by blocking NKCC2, induce a strong increase in urinary water, salt, and Ca^{2+} excretion.

The DCT is responsible for the reabsorption of ~5–10% of the filtered load of NaCl [4]. Driven by

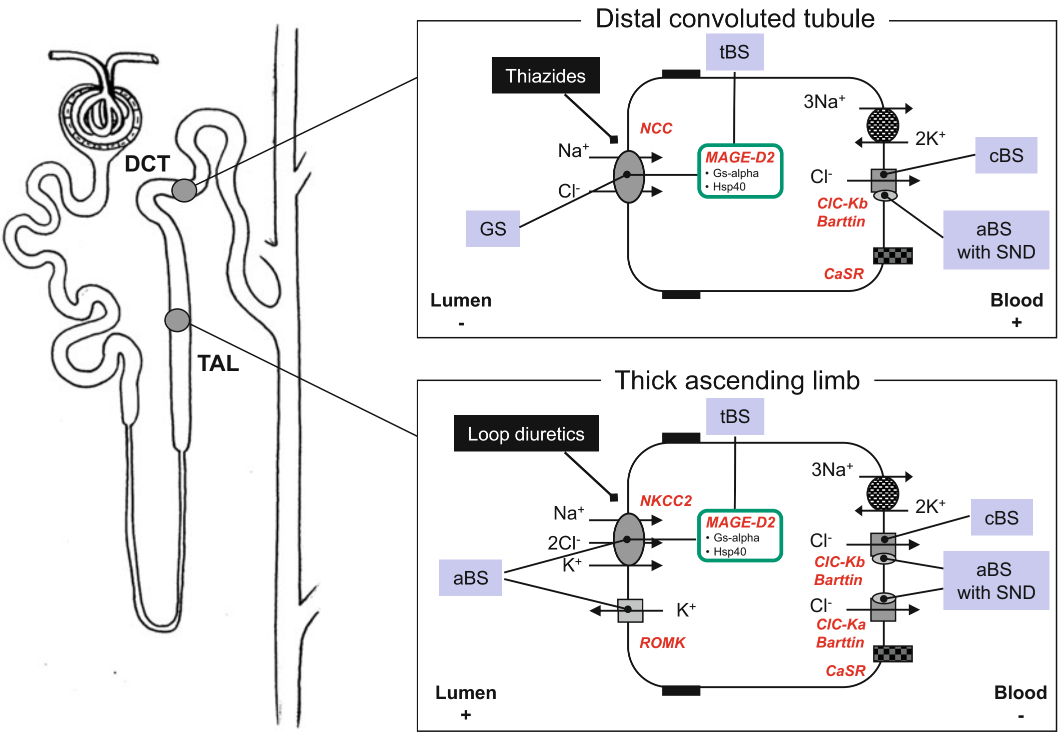
DCT
TAL
Distal convoluted tubule
tBS
Thiazides
3Na⁺
NCC
Na⁺
MAGE-D2
• Gs-alpha
• Hsp40
2K⁺
cBS
Cl⁻
Cl⁻
ClC-Kb
Barttin
GS
aBS
with SND
CaSR
Lumen
-
Blood
+
Thick ascending limb
tBS
Loop diuretics
3Na⁺
NKCC2
2K⁺
cBS
Na⁺
MAGE-D2
• Gs-alpha
• Hsp40
2Cl⁻
Cl⁻
K⁺
ClC-Kb
Barttin
aBS
aBS
with SND
K⁺
Cl⁻
ClC-Ka
Barttin
ROMK
CaSR
Lumen
+
Blood
-

Fig. 1 Molecular basis of Bartter syndromes and Bartter-like phenotypes. Approximately 25% of the filtered NaCl is reabsorbed in the thick ascending limb (TAL) via the apical Na^{+}-K^{+}-2Cl^{-} cotransporter NKCC2 (inhibited by loop diuretics), organized in parallel with the apical K^{+} channel ROMK to ensure K^{+} recycling and the lumen-positive voltage. The Na^{+}-K^{+}-ATPase and the Cl^{-} channels ClC-Ka and ClC-Kb associated with the regulatory β-subunit Barttin mediate the exit of Na^{+} and Cl^{-} ions from the cells. The thiazide-sensitive Na^{+}-Cl^{-} cotransporter NCC mediates 5–10% of the NaCl reabsorption in the distal convoluted tubule (DCT). Loss-of-function mutations in *SLC12A1* (coding for NKCC2) and *KCNJ1* (coding for ROMK) cause antenatal Bartter syndrome (aBS), whereas inactivating mutations of *BSND* encoding the beta-subunit barttin cause antenatal Bartter syndrome with sensorineural deafness (aBS with SND) and mutations in *CLCNKB* (ClC-Kb) cause classic Bartter syndrome (cBS). Inactivacting mutations of MAGED2 gene encoding MAGE-D2 cause transient antenatal Bartter syndrome (coding for NCC) are associated with Gitelman syndrome (GS). A few patients with autosomal dominant hypocalcemia due to severe gain-of-function mutations of the calcium-sensing receptor (CASR) may present a salt-losing, Bartter-like tubulopathy

the basolateral Na^{+}-K^{+}-ATPase, Na^{+} enters the DCT cells via the thiazide-sensitive Na^{+}-Cl^{-} cotransporter, NCC (or TSC, for thiazide-sensitive cotransporter). Because it is coupled to Na^{+}, Cl^{-} moves into the cell against its electrochemical gradient and then exits through the basolateral ClC-Kb channel. The DCT cells also secrete K^{+} via the ROMK channel and a K^{+}-Cl^{-} cotransporter located in the apical membrane and mediate the transcellular reabsorption of Ca^{2+} and Mg^{2+}, via the TRPV5/6 and TRPM6 channels, respectively, which belong to the transient receptor potential (TRP) channel family [5]. Thiazide diuretics, which inhibit NCC, induce a milder diuretic response than loop diuretics, typically associated with Mg^{2+} wasting and hypocalciuria.

The Bartter-like syndromes were initially classified into two major groups based primarily on age of presentation: the *antenatal Bartter syndrome* (aBS) (also named hyperprostaglandin-E syndrome (HPS), which can be transient or associated or not with sensorineural deafness (SND), and the *classic Bartter and Gitelman syndromes* (cBS and GS, respectively). The syndromes can also be classified based on their mechanism, albeit with some overlapping features. The aBS group includes disorders affecting the TAL, with furosemide-like manifestations, whereas the second group – and GS in particular – is due to a defect in the DCT, with thiazide-like manifestations [2]. From 1996, seminal studies by Lifton and colleagues identified loss-of-function mutations in transporters and channels responsible for these inherited tubulopathies. The aBS group was associated with inactivating mutations in the genes coding for NKCC2 [6] and ROMK [7], whereas inactivating mutations in barttin were detected in aBS with SND [8]. More recently, loss-of-function mutations in the melanoma-associated antigen-D2 (*MAGED2*), expressed in the TAL and DCT, were associated with a transient aBS (tBS) that spontaneously resolves during the first few months of postnatal life [9, 10]. On the other hand, inactivating mutations of ClC-Kb, also located both in the TAL and DCT, are associated with cBS [11], whereas GS is associated with mutations of NCC in the DCT [12] (Fig. 1).

A classification of these salt-losing tubulopathies, based on the clinical, physiological, and molecular insights discussed above, provides a basis to understand the phenotypes of these disorders (Table 1). When discussing such patients, the clinical diagnosis, based on relatively simple clinical criteria, should be complemented whenever possible by the genotyping information since there is only weak genotype-phenotype correlation in these disorders [13, 14].

Antenatal Bartter Syndrome

Genetics

Antenatal Bartter syndrome (aBS, MIM #601678, #241200) is a rare, life-threatening disorder characterized by massive polyuria that manifests in

Table 1 Inherited salt-losing tubulopathies

Disorder	OMIM	Inheritance	Gene locus	Gene	Protein	Affected tubular segment
Antenatal Bartter syndrome (aBS), hyperprostaglandin-E syndrome (HPS), type I Bartter syndrome[a]	#601678	AR	15q15-q21.1	*SLC12A1*	Na^+–K^+–$2Cl^-$ cotransporter NKCC2	TAL
Antenatal Bartter syndrome (aBS), hyperprostaglandin-E syndrome (HPS), type II Bartter syndrome[a]	#241200	AR	11q24	*KCNJ1*	K^+ channel ROMK (Kir1.1)	TAL + CCD
Antenatal Bartter syndrome with sensorineural deafness (aBS with SND)[b], type IV Bartter syndrome[a]	#602522	AR	1p31	*BSND*	Barttin, beta-subunit of ClC-Ka/b	TAL + DCT
Classic Bartter syndrome (cBS), type III Bartter syndrome[a]	#607364	AR	1p36	*CLCNKB*	Cl^- channel ClC-Kb	TAL + DCT
Transient antenatal BS, type V	#300971	XLR	Xp11.21	*MAGED2*	Melanoma antigen, family member D2 (MAGE-D2)	TAL + DCT
Gitelman syndrome (GS)	#263800	AR	16q13	*SLC12A3*	Na^+–Cl^- cotransporter NCC	DCT

TAL, thick ascending limb; *CCD*, cortical collecting duct; *DCT*, distal convoluted tubule; *AR*, autosomal recessive, *XLR* X-linked recessive

[a] This classification is based on the chronological order of gene discovery

[b] A digenic disorder with inactivating mutations of *CLCNKA* and *CLCNKB* has been associated with the aBS with SND phenotype

utero by polyhydramnios and premature delivery in almost all cases. Affected neonates develop salt wasting, hypokalemic metabolic alkalosis, and profound polyuria [15–17]. The disorder is accompanied by markedly elevated urinary prostaglandin E2 (PGE_2) excretion: treatment with PG synthesis inhibitors effectively reduces clinical and biochemical manifestations. The latter features explain why aBS has also been designed as hyperprostaglandin-E syndrome (HPS) [18]. As patients with aBS/HPS fail to respond to loop diuretics, a defective NaCl reabsorption in the TAL was suspected [19]. By combining a candidate gene approach with linkage analysis, Simon et al. demonstrated that aBS/HPS is due to mutations either in NKCC2 (type I BS) or in ROMK (type II BS) [6, 7] (Fig. 1). The two forms of aBS are clinically and biochemically almost indistinguishable [20].

Type I BS is due to mutations in the *SLC12A1* gene located on 15q15-q21.1 and containing 26 exons [6, 17, 21]. The *SLC12A1* gene codes for the bumetanide-sensitive NKCC2, a 121 kD protein with 12 putative membrane-spanning domains. NKCC2 is expressed in the apical membrane of epithelial cells lining the TAL and in the macula densa [22]. Loop diuretics bind to portions of transmembrane domains 11 and 12, whereas portions of domains 2, 4, and 7 are involved in ion transport [23]. Dozens of mutations in *SLC12A1* have been reported, essentially missense or frameshift [24]. Aberrant exon skipping was recently reported as a previously unrecognized mechanism by which an exonic variant in *SLC12A1* can lead to BS type I [25]. The five initial kindreds [6] and others reported subsequently [17, 21] were consanguineous, but compound heterozygotes and patients harboring only one heterozygous mutation have been reported [17, 21, 24, 26]. Although a founder mutant allele (W625X) was reported in a cohort of Costa Rican patients [26], the aBS mutations are evenly distributed throughout the *SLC12A1* gene [24]. Of note, alternative splicing of the NKCC2 pre-mRNA results in the formation of three full-length isoforms of NKCC2, which differ in their variable exon 4, their localization along the TAL, and their transport characteristics [27]. Mutations affecting the low-capacity/high-affinity isoform could thus result in a milder phenotype [17]. Novel mutations in *SLC12A1* have recently been identified by whole exome sequencing in patients with atypical type I BS presenting with varying degrees of hypercalcemia and primary hyperparathyroidism [28–30].

Type II BS is due to mutations in the *KCNJ1* gene that is located on chromosome 11q24 and contains five exons [7]. The *KCNJ1* gene encodes ROMK (also known as Kir1.1), a luminal ATP-sensitive, inwardly rectifying K^+ channel that is critical for K^+ recycling in the TAL and constitutive K^+ secretion in the distal nephron [31]. ROMK channels are assembled from four subunits, each consisting of two transmembrane domains flanking a conserved loop that contributes to the pore and selectivity filter and cytoplasmic N^- and C^- termini that contain regulatory and oligomerization domains [32]. ROMK exists in three N-terminal splice variations that all behave as rectifying K^+ channels gated by intracellular pH [31]. Through the recycling of reabsorbed K^+ back to the lumen, ROMK is believed to be a regulator of NKCC2 cotransporter activity. Therefore, loss of function in ROMK, as well as in NKCC2, disrupts NaCl reabsorption in the TAL. Mutations in *KCNJ1* that cosegregate with aBS include missense, nonsense, frameshift, and deletions [7], [20, 33–37]. The first mutations were reported in exon 5, common to all ROMK isoforms [7, 20], but homozygous deletions in exons 1 and 2 have also been reported [33, 36].

Clinical Manifestations

Typical features of aBS type I (NKCC2) and type II (ROMK) include polyhydramnios (within the second trimester of gestation); premature delivery (usually around 32 weeks); severe polyuria; life-threatening episodes of dehydration; hypercalciuria, leading to nephrocalcinosis within the first months of life; and activation of the RAAS (Table 2). The polyuria can be massive (>20 mL/kg/h) despite adequate fluid replacement. Magnesium wasting is not common in aBS [38], although hypomagnesemia was evident in half of the Costa Rican patients [39]. Failure to thrive and growth retardation are invariably observed [16, 39, 40]. A peculiar facies, characterized by a

Table 2 Clinical and biochemical features of Bartter syndrome (BS) and Gitelman syndrome (GS)

Feature	Type I BS	Type II BS	Type IV BS	Type III BS	Type V BS	GS (*SLC12A3*)
Age of onset	Antenatal	Antenatal	Antenatal	Variable	Antenatal, transient	Childhood (> age 6 years)
Maternal polyhydramnios	Present	Present	Present	Rare	Present, very severe	Absent
Prematurity	Present	Present	Present	Rare	Present	Absent
Polyuria	Present	Present	Present	Occasional	Present	Absent
Failure to thrive	Present	Present	Present	Common	Present	Absent
Growth retardation	Present	Present	Present (severe)	Common		Occasional
Spasm/tetany/ muscle weakness	Absent	Absent	Absent	Occasional		Present
Nephrocalcinosis	Present	Present	Absent	Rare	Rare	Absent
Sensorineural deafness	Absent	Absent	Present	Absent[a]	Present	Absent
Hypokalemic metabolic alkalosis	Present	Present (transient neonatal hyperkalemia)	Present	Present	Present	Present
Plasma Mg^{2+}	Normal	Normal	Normal or low	Normal or low		Low
Urinary Ca^{2+} excretion	High	High	Moderate (transient) or normal	Usually normal	High	Low
Urinary NaCl excretion	High	High	Very high	Variable increase	High	Mild increase
Maximal urine osmolality	Hyposthenuria	Hyposthenuria	Iso-/ hyposthenuria	Usually normal		Normal
High renin/ aldosteronism	Present	Present	Present	Present	Present	Present
Urinary PGE2 excretion	High	High	High	High		Normal

[a] SND is present in case of digenic disorder with inactivating mutations of *CLCNKA* and *CLCNKB*

triangularly shaped face, prominent forehead, large eyes, protruding ears, and drooping mouth, has been reported but could reflect dystrophic premature babies [27, 39]. Systemic manifestations including fever of unknown origin, diarrhea, vomiting, and generalized convulsions, which have been attributed to enhanced systemic overproduction of PGE, and recurrent urinary tract infection may occur [38, 41]. Osteopenia is common in aBS [16, 42], associated with high urinary excretion of bone resorption markers [43]. Increased urinary PGE2 excretion is usually detected, although not invariably [2, 16]. Hypophosphatemia with decreased tubular phosphate reabsorption has been described, possibly related to tubular damage and hypokalemic nephropathy [40, 43]. High Cl^- and aldosterone concentrations in the amniotic fluid have been reported [16].

Some phenotype variability among NKCC2-deficient patients has been reported, including absence of hypokalemia and/or metabolic alkalosis during the first years of life and persistent metabolic acidosis or hypernatremia [21]. The Costa Rican cohort harboring the W625X founder allele showed a somewhat milder phenotype with a median age of diagnosis at 10 months of life and no necessity of indomethacin treatment in most patients [26, 39]. A late-onset presentation (age

13 and 15 years) with mild polyuria and borderline hypercalciuria has been reported in two compound heterozygote brothers for NKCC2 mutations [44].

While kidney function is generally well preserved in aBS [21, 39], chronic kidney disease (CKD) leading to ESRD has been reported [39, 45, 46]. The potential mechanisms that could lead to kidney damage in aBS include consequences of early neonatal events and dehydration episodes, hypokalemic nephropathy, nephrocalcinosis, and potential nephrotoxicity of nonsteroidal anti-inflammatory drugs (NSAID) [45, 47, 48]. Kidney biopsies of children with aBS revealed a marked hypertrophy and hyperplasia of the juxtaglomerular apparatus, with stimulation of the renin-angiotensin system [49, 50]. This feature is not specific for aBS and may be observed in all Bartter-like syndromes. Reinalter et al. [48] showed inflammatory infiltrates, with areas of interstitial fibrosis, focal tubular atrophy with thickening of basement membranes and degenerated tubular epithelia, and focal segmental mesangial matrix increase and hypercellularity in kidney biopsies obtained in 10aBS/HPS patients. Of interest, patients harboring ROMK mutations had only minimal histological lesions as compared with NKCC2-deficient patients and cBS patients [48].

Because NKCC2 and ROMK are functionally coupled in the apical membrane of the TAL, patients with defective ROMK have a very similar clinical picture to NKCC2 deficiency, with polyhydramnios, premature delivery, severe neonatal polyuria with isosthenuria, and hypercalciuria with secondary nephrocalcinosis [2, 38]. However, there is an important difference in that ROMK-deficient patients show a transient hyperkalemia during the first days of life, correlated with gestational age [38, 51]. The association of early hyperkalemia with hyponatremia and hyperreninemic hyperaldosteronism may erroneously suggest the diagnosis of pseudohypoaldosteronism type 1 (PHA1) [51]. Potassium wasting in these cases may not be apparent until 3–6 weeks postnatally, leading to modest hypokalemia in most patients. Typically, hypokalemia in ROMK-deficient patients is less severe than that observed in NKCC2 patients [36, 38].

By exome sequencing approximately 3000 subjects in the Framingham Heart Study (FHS) cohort, Ji et al. showed that heterozygote carriers of inactivating mutations in NKCC2 and ROMK (as well as NCC) had significantly lower systolic and diastolic BP and a significant reduction in the risk of developing hypertension [52]. It was subsequently demonstrated that polymorphisms in *KCNJ1* were associated with BP values in a cohort of 2037 adults after adjusting for age, sex, and familial correlations [53]. Since the vast majority of these rare variants in NKCC2 and ROMK have a loss-of-function effect, it can be concluded that such rare variants may exert a profound influence on BP regulation [54, 55].

Differential Diagnosis

Antenatal BS should always be suspected in the face of polyhydramnios due to fetal polyuria. Patients with type II aBS due to ROMK deficiency may show a transient hyperkalemia, which can mimic PHA1. However, PHA1 is characterized by permanent hyperkalemia with metabolic acidosis, whereas type II aBS patients typically have metabolic alkalosis, as well as hypercalciuria and nephrocalcinosis. In some patients with aBS, the urinary concentrating defect is so severe that it can lead to hypernatremia, resembling nephrogenic diabetes insipidus [21]. Some patients with aBS may lack metabolic alkalosis during the first year of life or even present a transient metabolic acidosis with defective urinary acidification [56]. This association, which probably results from medullary nephrocalcinosis, can mimic incomplete distal renal tubular acidosis [21]. Other causes of pseudo-Bartter syndromes will be discussed in the section "Classic Bartter Syndrome."

Pathophysiology

Functional investigations of pathogenic NKCC2 mutations in *Xenopus laevis* oocytes revealed a low expression of normally routed but functionally impaired transporters [57]. Reduced transport function, reduced Na^+ affinity, and defective processing were also observed when expressing rare NKCC2 variants associated with low BP in the FHS [58, 59]. A partial intrinsic transport defect was demonstrated in the F177Y mutant, which may account for the attenuated phenotype of the affected patients [44]. Similar studies provided insights into the role of ROMK in aBS. Two C-terminal mutations located nearby or inside the putative protein kinase A (PKA) phosphorylation site of ROMK showed a decreased open probability of the channel [60]. Missense mutations located in the intracellular N- and C-termini were shown to encode functional channels, but with altered pH gating [35]. Starremans et al. investigated eight ROMK mutants and showed that loss of function may result from defective cellular routing to the plasma membrane or impaired channel function [37]. Peters et al. [61] identified defective membrane trafficking in 14/20 naturally occurring ROMK mutations and showed that two early inframe stop mutations could be rescued by aminoglycosides, resulting in full-length ROMK and correct trafficking to the plasma membrane. More recently, type II BS mutations in ROMK have been associated with accelerated endoplasmic reticulum (ER)-associated degradation (ERAD) [62]. Rare variants of ROMK associated with low BP in the FHS also showed reduced ROMK channel activity by different mechanisms [63].

The functional data obtained in vitro suggest that loss-of-function mutations in NKCC2 and ROMK disrupt NaCl transport in the TAL, leading to salt wasting, volume contraction, and stimulation of the RAAS. In turn, enhanced Na^+ delivery to the distal nephron and reabsorption of Na^+ via the Na^+ channel ENaC stimulate urinary excretion of K^+ and H^+, causing hypokalemic metabolic alkalosis (Fig. 2). These changes are already observed prenatally, with polyhydramnios, high Cl^- concentrations, and increased aldosterone levels in the amniotic fluid [64, 65]. Chronic volume contraction, elevated levels of angiotensin II, and intracellular Cl^- depletion stimulate PGE2 production, which further inhibits NaCl transport in the TAL and contributes to the washout of the osmotic gradient through enhanced medullary perfusion [2]. The inhibition of NKCC2 in the macula densa could impair the luminal Cl^- sensing of these cells, which may disrupt the tubuloglomerular feedback and further activate renin release from juxtaglomerular cells [66]. It also appears that cyclooxygenase-2 (COX-2) is induced in the macula densa of children with aBS [67], which could further contribute to hyperreninemia [68]. The early neonatal hyperkalemia harbored by some ROMK-deficient patients is explained by the role of ROMK in K^+ secretion in the aldosterone-sensitive distal nephron (ASDN). The transient nature of the hyperkalemia [51]

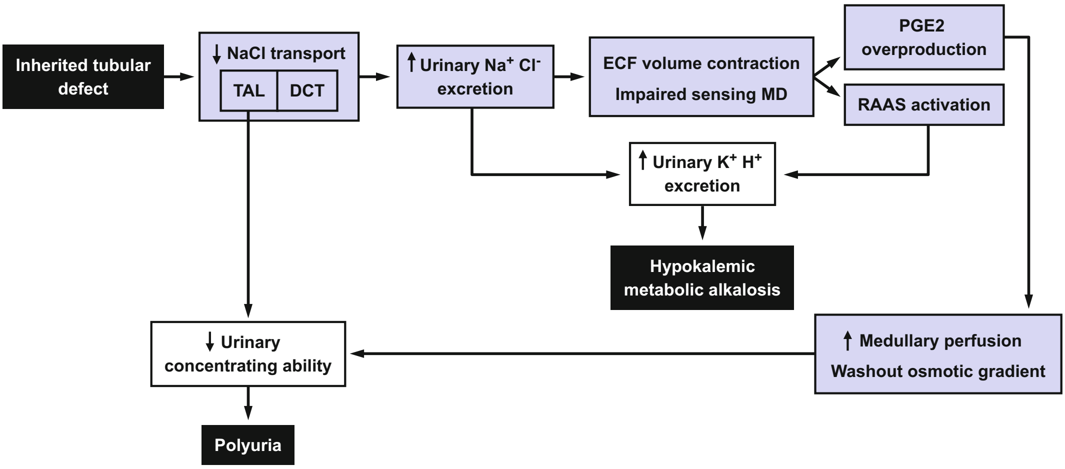

Fig. 2 Physiopathology of hypokalemic metabolic alkalosis and polyuria in Bartter syndrome. See text for details. *Abbreviations*. TAL, the thick ascending limb; DCT, distal convoluted tubule; MD, macula densa; RAAS, renin-angiotensin-aldosterone system

likely reflects the postnatal appearance and activation of other ASDN K^+ secretory channels, notably the BK channel, that can compensate for the absence of ROMK [69, 70].

The impaired Cl^- transport in the TAL affects the lumen-positive electrical potential, causing persistent hypercalciuria and early-onset nephrocalcinosis in aBS. Magnesium wasting and overt hypomagnesemia do not reliably segregate with aBS, consistent with the observation that chronic furosemide treatment is not generally associated with hypomagnesemia [71]. Possibly, the loop of Henle or more distal segments may adapt and compensate more efficiently for Mg^{2+} than Ca^{2+} in this syndrome. In the TAL, adaptation might involve tight junction proteins (claudin16/claudin19), whereas increased PGE2 synthesis may contribute to increased Mg^{2+} reabsorption in the DCT [72]. It has been suggested that bone resorption and PGE2-mediated increase in calcitriol may contribute to hypercalciuria in aBS [42, 73]. Schurman et al. [74] showed that elevated levels of angiotensin II may stimulate the release of basic fibroblast growth factor (b-FGF), with a resulting increase in bone resorption via a prostaglandin-dependent mechanism.

NKCC2 knockout (KO) mice show massive perinatal fluid wasting and dehydration, leading to kidney failure and death prior to weaning [75]. Treatment of these mice with indomethacin from day 1 allowed 10% mice to survive, despite polyuria, hydronephrosis, hypokalemia, and hypercalciuria. Similarly, the ROMK KO mice manifest early death associated with polyuria, polydipsia, and impaired urinary concentrating ability [76]. Approximately 5% of these mice survive the perinatal period but show kidney failure, hypernatremia, and metabolic acidosis. Micropuncture analysis revealed that the absorption of NaCl in the TAL was reduced, with severe impairment of the tubuloglomerular feedback. Another strain of ROMK-null mice with a BS-like phenotype showed increased survival to adulthood, due to compensatory mechanisms mostly active in the DCT [77]. Lu et al. demonstrated that the CFTR Cl^- channel may regulate the ATP sensitivity of ROMK in the TAL, which could explain why patients with cystic fibrosis are prone to develop the pseudo-Bartter features of hypokalemic metabolic alkalosis [78].

Treatment

The initial treatment of preterm infants or neonates with aBS should focus on the correction of dehydration and electrolyte disorders, which often requires continuous saline infusion in a neonatal intensive care unit. Elevated levels of urinary PGE2 provided a rationale for NSAIDs, and indomethacin has been widely used [2, 16, 38, 40, 56]. Typically, administration of indomethacin starting 4–6 weeks after birth, when massive urinary electrolyte losses have been controlled and hypokalemic metabolic alkalosis is established, corrects both the systemic and biochemical manifestations of aBS [38]. Indomethacin (at doses ranging from 0.5 mg/kg/day to 2.5 mg/kg/day) reduces polyuria, improves hypokalemia, normalizes plasma renin levels, and reduces hypercalciuria [16, 38, 40, 41, 56]. However, the potential benefit of indomethacin administration in premature babies and neonates should be weighed against potential risks of severe gastrointestinal complications, such as ulcers, perforation, and necrotizing enterocolitis [16, 40, 79]. In particular, administration of indomethacin in newborn infants with defective ROMK may be complicated by oliguric kidney failure and severe hyperkalemia. At any time, the ROMK-deficient patients are particularly sensitive to indomethacin, with doses well below 1 mg/kg/day sufficient to maintain normal plasma K^+ levels [80]. Similarly, the potential benefit of prenatal treatment with indomethacin [80] should be weighed against the lack of evidence for hyperprostaglandinism in the fetus [16] and the negative effects of NSAID on the ductus arteriosus and the development of the kidney [79]. Selective and nonselective cyclooxygenase inhibitors can also be used for treatment [80]. As an alternative to indomethacin, the COX-2 selective inhibitor rofecoxib ameliorated clinical and biological manifestations in aBS patients, with significant suppression of PGE2 and correction of hyperreninemia [68, 81]. However, a case of reversible acute renal failure associated with rofecoxib in an 18-month-old girl with aBS has been reported [82].

Importantly, it should be remembered that HPS is secondary to volume depletion [16] and that the appropriate compensation for fluid and salt wasting remains the essential priority. Additional K^+ supplementation is required, more often for NKCC2-deficient than ROMK-deficient patients [2, 38]. In some cases, a K^+-sparing diuretic (e.g., spironolactone) is necessary to increase serum K^+ levels [1, 16]. Treatment with angiotensin-converting enzyme (ACE) inhibitors has been reported effective in a few cases [40, 83] but should be used with caution as these drugs could block the distal compensatory Na^+ reabsorption. Thiazides should not be used to reduce hypercalciuria, since they interfere with compensatory mechanisms in the DCT and further aggravate dehydration.

The appropriate management of aBS with correction of fluid and electrolyte disorders, indomethacin, and K^+ supplements results in catch-up growth and normal pubertal and intellectual development [16, 40]. However, most patients show a persistent deficiency in height and weight [39, 48]. Correction of hypercalciuria is usually partial, with progression of nephrocalcinosis and development of CKD in some cases [16, 40, 45, 56]. Chaudhuri et al. [46] reported a case of severe aBS in whom pre-emptive nephrectomy followed by a living-related donor kidney transplantation resulted in correction of metabolic abnormalities and excellent graft function.

Few long-term follow-up studies of patients with BS have been published. A long-term follow-up study of 15 children with homozygous or compound heterozygous mutations in *SLC12A1* or *KCNJ1* by Puricelli et al. [84] revealed that in response to potassium chloride supplementation and indomethacin treatment, patients presented a satisfactory somatic growth after a median follow-up of more than 10 years. An estimated glomerular filtration rate (eGFR) of <90 ml/min/1.73 m^2, occasionally associated with overt proteinuria, was present at follow-up in 25% of the patients, which can be possibly explained by nephrocalcinosis and/or prolonged hypokalemia or by the side effect of the NSAIDs. Of note, 3/15 patients developed gallstones which might represent a new complication of aBS [84]. In another long-term follow-up study (median 8.9 years, between 1984 and 2014) of 45 children with pathogenic variants in *SLC12A1* ($n = 8$), *KCNJ1* ($n = 8$), *CLCNKB* ($n = 17$), *BSND* ($n = 2$), and *SLC12A3* ($n = 10$), impairments at the last follow-up were found in growth, albeit within the normal range (median height standard deviation score -1.2) and eGFR <90 mL/min/1.73 m^2, predominantly with *SLC12A1* and *KCNJ1* mutations [85]. Pathological albuminuria was detected in 31 of the 45 children. These observations underscore the critical need that children diagnosed with BS are closely monitored for glomerular and tubular function.

Transient Antenatal Bartter Syndrome (tBS)

Genetics

In 2013, Laghmani et al. [9] described a very severe but transient BS characterized by the very early onset of extreme polyhydramnios, prematurity, and perinatal mortality. Whole exome sequencing of an affected male infant and his mother in the index family, in which the male offspring of three pregnancies presented with this severe phenotype, identified a truncating mutation in the *MAGED2* gene [9]. *MAGED2* maps to the X chromosome and encodes the melanoma-associated antigen D2 (MAGE-D2) protein [86]. Sequencing of additional families with affected male infants with similar clinical presentations and women with recurrent idiopathic polyhydramnios during pregnancies with male fetuses confirmed the presence of at least 15 *MAGED2* mutations that cosegregate with disease [9, 10]. The identification of two female patients in a French cohort was proposed to reflect selective inactivation of the X chromosome [10]. Mutations in *MAGED2* associated with aBS include missense, nonsense, splicing, frameshift, and deletions [9, 10]. This subtype of aBS has been named type V transient aBS (MIM #300971) (Fig. 1).

Clinical Manifestations

Patients harboring mutations in *MAGED2* show the most severe form of aBS, with maternal polyhydramnios beginning by week 20 of gestation, necessitating serial amnioreductions and in some cases antenatal indomethacin treatment [10], and severe prematurity, with ~40% affected fetuses born at <28 weeks GA [9], [10]. Polyuria is severe and mortality is high [9], [10]. Patients with *MAGED2* mutations can be distinguished from other aBS groups by their higher birth weight percentile for GA and higher plasma Cl^- concentration [10]. They present with lower serum bicarbonate values compared to other subjects with aBS, suggesting that MAGE-D2 also affects bicarbonate transport. Unlike the other forms of BS, salt and water losses in transient aBS spontaneously resolve typically between 2 and 18 months of age [9, 10].

Pathophysiology

MAGE-D2 is expressed in the TAL and DCT of both fetal and adult kidneys [9]. Immunohistochemical analyses of the kidney of a stillborn fetus carrying a *MAGED2* mutation in the index family reported by Laghmani [9] revealed a weak apical signal for NKCC2 and NCC, with protein predominantly cytoplasmic, colocalizing to the endoplasmic reticulum (ER) [9]. These observations are consistent with the Na^+ wasting and polyuria in affected infants and suggest that MAGE-D2 participates in maturation of proteins in the ER for transport to the apical membrane.

Using a proteomic approach applied to a heterologous expression system, Laghmani et al. [9] showed that wild-type but not mutant MAGE-D2 binds to the chaperone protein Hsp40 (DNAJB1) and the G protein subunit Gs-alpha (GNAS). Hsp40 modulates protein folding and stability, especially under ER stress conditions, and regulates NCC biosynthesis in the ER [88]. Laghmani et al. [9] showed that MAGE-D2 protects NKCC2 and NCC from ER-associated degradation (ERAD), promoting total and cell surface expression and activity of the transmembrane proteins. Thus, Hsp40 has been proposed to interact with MAGE-D2 to regulate ERAD of NKCC and NCC. Activation of Gs-alpha enhances generation of cAMP as well as surface expression, phosphorylation, and activity of NKCC2 and NCC [89, 90]. These observations, in light of the finding that knockdown of *GNAS* in mice reduces NKCC2 protein abundance [91], suggest that MAGE-D2 modulates trafficking of these transporters to the cell surface. In sum, loss-of-function mutations in MAGE-D2 are predicted to inhibit NKCC2 and NCC expression and activity. The severity of this form of aBS reflects the dual effects on the major Na^+ absorptive transport pathways in the TALH and DCT.

The transient nature of the disease may reflect (i) a maturational increase in sensitivity of adenylate cyclase to vasopressin as activity of phosphodiesterase, which catalyzes the degradation of cAMP, declines [92, 93] which compensates for the low Gs-alpha activity to enhance cAMP generation, and/or (ii) higher levels of oxygenation with advancing pregnancy/delivery may reduce hypoxia-induced ERAD and thus protect Hsp40-modulated NKCC2 and NCC biogenesis, thereby reducing the need for MAGE-D2 [9, 94].

Treatment

The treatment for tBS is similar to that described above for **antenatal Bartter syndrome**, recognizing that the kidney phenotype of type V BS is transient and resolves spontaneously. A genetic diagnosis and close clinical follow-up should ensure that the patient is not subject to overtreatment with NSAIDs with potential toxicity.

Antenatal Bartter Syndrome with Sensorineural Deafness

Genetics

In 1995, Landau et al. [95] described a subtype of aBS/HPS associated with sensorineural deafness (SND) in five affected subjects from an inbred Bedouin kindred. These patients had severe salt wasting and fluid loss, with poor response to indomethacin and, most often, CKD [95, 96]. By studying the original kindred, Brennan et al. mapped the disease-causing gene to chromosome 1p31 [97]. In 2001, Birkenhager et al. [8] identified a novel gene, *BSND*, within the critical interval and detected inactivating mutations in affected individuals. This subtype of aBS was named aBS with SND, or type IV BS (MIM #602522). The original mutations included a splicing mutant, a deletion of two exons, three missense mutations affecting a conserved residue close to the first putative membrane domain, and one mutation resulting in the loss of the start codon [8]. The *BSND* gene consists of four exons. It encodes barttin, a 320 amino acid protein that contains two putative transmembrane domains and serves as an essential subunit of the basolateral Cl^- channels ClC-Ka and ClC-Kb. Barttin is expressed in the thin limb and TAL and in the DCT (Fig. 1) and in the stria vascularis surrounding the cochlear duct in the inner ear [8, 98]. BS type IVa is due to mutations in *BSND*, whereas the phenotypically indistinguishable BS type IVb is caused by mutations in the genes encoding both basolateral Cl^- channels. As Cl^- efflux is necessary for depolarization of hair cells in the inner ear, loss of function of barttin and/or both Cl^- channels in BS IV (vs. BS III where ClC-Ka remains intact) is associated with deafness.

Clinical Manifestations

Typically, patients harboring mutations in *BSND* show a severe form of aBS, with maternal polyhydramnios beginning at week 25 of gestation, severe prematurity, life-threatening neonatal episodes of dehydration, polyria with hypo- or isosthenuria, and increased urinary PGE2 excretion [2, 99] (Table 2). The patients are deaf and show a severe growth defect, with delayed motor development [96]. They present multiple episodes of fever, vomiting, and bacterial infections. Jeck et al. [96] reported progressive CKD in all patients, attributable to glomerular sclerosis and tubular atrophy. However, Shalev et al. reported that early kidney failure is not a uniform finding [99]. In contrast with patients harboring mutations in NKCC2 and ROMK, barttin-deficient patients exhibit only moderate and transient hypercalciuria and do not show nephrocalcinosis [2, 99]. This could be due to defective NaCl transport in both the TAL and DCT, with divergent effects on urinary calcium excretion somehow similar to a combined action of a loop diuretic with a thiazide. Accordingly, barttin-deficient patients may show a severe Mg^{2+} wasting, caused by a defect in both the paracellular (TAL) and transcellular (DCT) pathways of Mg^{2+} reabsorption [2]. Of note, a lack of diuretic response to furosemide and to hydrochlorothiazide was evidenced in one barttin-deficient patient, supporting a defect in both TAL and DCT [100].

There is some degree of phenotype variability among patients with *BSND* mutations. Miyamura et al. [101] reported a patient harboring the loss-of-function G47R mutation of *BSND* who presented at age 28, with congenital deafness but without polyhydramnios, premature labor, or severe salt wasting in the neonatal period. In contrast, five patients from two unrelated Spanish families harboring the same G47R mutation presented with polyhydramnios, premature birth, and salt loss [102]. Kitanaka et al. [103] reported a patient harboring two mutations in *BSND* (Q32X and G47R) who presented with relatively mild perinatal clinical features but developed end-stage kidney failure at age 15, requiring kidney transplantation. The functional evaluation of the G210S mutation of *BSND* revealed only a very mild disturbance in the current-voltage relationship [104], possibly accounting for a milder phenotype [99]. Riazuddin et al. identified a missense mutation (I12T) of *BSND* in four kindreds, associated with nonsyndromic deafness. Of note, the I12T mutation does not affect the Cl^- channel function and only interferes with the chaperone function of barttin in intracellular trafficking [105]. Functional studies of six *BSND* mutations have revealed that missense mutations can affect the function of ClC-K/barttin despite normal trafficking and membrane insertion of the channels or by binding less efficiently to ClC-K, whereas nonsense mutants interfere with the apical surface sorting [106].

Kidney biopsies obtained in patient with *BSND* mutation showed variable features including hyperplasia of the juxtaglomerular apparatus, mild mesangial hypercellularity, mild to severe tubulointerstitial fibrosis, areas of tubular atrophy, and sclerosed glomeruli [96, 99].

Pathophysiology

Functional expression studies revealed that barttin is an essential beta-subunit for the Cl^- channels ClC-Ka and ClC-Kb, by stimulating Cl^- currents and enhancing surface expression of these channels [98]. Barttin stimulates Cl^- flux through the human ClC-Ka by modifying the gating of these double-barreled channels [107]. ClC-Ka and ClC-Kb are two members of the ClC gene family that are located on the basolateral membrane of the cells lining the thin ascending limb (ClC-Ka only), TAL and DCT, and the intercalated cells of the collecting duct. ClC-Ka and ClC-Kb are also expressed in the inner ear, where they colocalize precisely with barttin in specialized, K^+-secreting cells of the stria vascularis and the vestibular organ [98]. The co-expression of barttin with ClC-Ka/b channels is crucial for NaCl reabsorption in the TAL/DCT (Fig. 1) and K^+ recycling in the inner ear. Disease-causing mutations in barttin disrupt the Cl^- exit from the TAL and DCT, causing the severe salt-losing tubulopathy. Furthermore, the defective barttin-ClC-Ka/b complex impairs the basolateral recycling of Cl^- in the stria vascularis, decreasing the secretion of K^+ into the endolymph and causing SND [98, 108]. The role of barttin as an essential beta-subunit for ClC-Ka and ClC-Kb has been substantiated by reports of patients showing a typical aBS with SND phenotype indistinguishable from barttin-deficient patients, but due to a digenic disease caused by loss-of-function mutations in both *CLCNKB* and *CLCNKA* genes [109, 110].

A barttin KO mouse model was previously generated, but the mice died within days after birth because of severe dehydration [111]. Nomura et al. generated a knockin mouse model carrying the disease-causing R8L mutation of *BSND* [112]. These mice presented with hypokalemia, metabolic alkalosis, and decreased distal NaCl reabsorption under a low-salt diet. Plasma membrane localization of both mutant barttin and the ClC-K channel was impaired as demonstrated by immunofluorescence and immunoelectron microscopy, and transepithelial chloride transport in the thin ascending limb of Henle's loop as well as thiazide-sensitive chloride clearance was significantly reduced. These changes were correlated with the reduced quantity of mutant barttin localized to the plasma membrane [112].

Treatment

Barttin-deficient patients are managed primarily with intravenous fluids in neonatal intensive care units. In contrast with other forms of aBS, and despite high levels of urinary PGE2, the effect of indomethacin on growth and correction of electrolyte disorders is rather poor [96, 99]. Hypokalemic metabolic alkalosis persists despite high doses of NaCl and KCl supplementation [96]. Zaffanello et al. [100] reported that combined therapy with indomethacin and captopril was needed to discontinue intravenous fluids and improve weight gain in a single patient. A preemptive nephrectomy for refractory electrolyte and fluid losses and persistent failure to thrive, followed by peritoneal dialysis and successful kidney transplantation, has been reported in a 1-year-old child with type IV BS [46]. Recently, treatment with the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG), known to rescue ER-trapped mutant proteins in vitro, increased the plasma membrane expression of mutant barttin in the kidney and inner ear and improved the electrolyte disorders and hearing loss of the R8L knockin mouse model [113].

Classic Bartter Syndrome

Genetics

Classic Bartter syndrome (cBS, or type III BS, MIM #607364) usually presents during infancy or early childhood, with a phenotype similar to the original description given by Bartter et al. [1], i.e., without the prenatal onset and the nephrocalcinosis seen in the aBS variant. The cBS variant is caused by mutations in the *CLCNKB* gene located on 1p36 ([11, 114]. The gene, which contains 19 exons, encodes the basolateral Cl^- channel ClC-Kb, which mediates Cl^- efflux from epithelial cells lining the TAL and DCT [11, 108] (Fig. 1). There is a high rate of deletions encompassing a part of or the entire *CLCNKB* gene [11, 114]. It is hypothesized that the close vicinity of the almost identical *CLCNKA* and *CLCNKB* genes, which are separated by only 11 kb, predisposes to a high rate of rearrangements, for example, by unequal crossing over as demonstrated in two kindreds

[11, 114]. In addition, missense, nonsense, small insertions/deletions, frameshift, and splice-site mutations have also been reported (http://www.hgmd.cf.ac.uk) [115, 116]. A founder mutation (A204T) affecting a highly conserved residue has been reported at the homozygous state in ten patients from nine unrelated, non-consanguineous families in Spain [117]. As expected for a recessively transmitted disorder, a significant number of subjects originate from consanguineous kindred [11, 114].

Pathophysiology

As mentioned earlier (see Pathophysiology section of "Antenatal Bartter Syndrome with Sensorineural Deafness"), ClC-Kb is a plasma membrane channel that belongs to the CLC family of chloride channels/exchangers [108]. ClC-Kb and the closely related ClC-Ka isoform are located on the basolateral membrane of the cells lining the thin ascending limb (ClC-Ka only), TAL and DCT cells, as well as in the intercalated cells of the collecting duct (Fig. 1). They both require the beta-subunit barttin to facilitate their insertion in the plasma membrane and generate Cl^- currents [98, 104]. Disease-causing missense mutations of ClC-Kb result in significant reductions or the loss of ClC-Kb/barttin currents [98, 118]. Thus, inactivating mutations in ClC-Kb affect the basolateral exit of Cl^-, which in turn reduces the reabsorption of NaCl in the TAL and DCT. The phenotypic variability of type III BS, which ranges from aBS/HPS in some cases to typical GS in others, may be explained by the wide distribution of ClC-Kb [38, 114] and the fact that alternative pathways for Cl^- exit could partially compensate for ClC-Kb inactivation in the kidney [114, 119]. The nature of *CLCNKB* mutations may also play a role [118, 120]. Functional studies of nine *CLCNK*B mutations in *Xenopus laevis* oocytes revealed three classes of mutations: one characterized by impaired or reduced surface expression due to partial or total retention in the ER, a second characterized by reduced channel insertion at the plasma membrane and altered gating, and a novel third group of functionally inactive mutants expressed normally at the plasma membrane, consistent with a channel gating impairment [121, 118]. Importantly, none of the type III BS patients with ClC-Kb mutations is deaf, because the function of ClC-Kb/barttin channels in the inner ear can be replaced by ClC-Ka/barttin. Only the disruption of the common β-subunit barttin [98] or the combined loss of ClC-Ka and ClC-Kb [109, 110] results in a Cl^--recycling defect that lowers K^+ secretion in the stria vascularis to a pathogenic level.

Mice with targeted disruption of ClC-K2, the murine ortholog of ClC-Kb, develop a phenotype characterized by salt loss, marked hypokalemia, and metabolic alkalosis [122]. These KO mice failed to respond to furosemide and exhibited a blunted natriuretic response to thiazide, consistent with the critical role of this Cl^- channel in the TAL and ASDN [122]. Mice lacking ClC-K1, the murine ortholog of ClC-Ka, show a phenotype of nephrogenic diabetes insipidus, with no modification in the fractional excretion of Na^+ and Cl^- and no hypokalemic alkalosis [123]. These features are caused by the loss of Cl^- transport across the thin ascending limb, which is essential for generating a hypertonic interstitium [123]. No corresponding human disease linked to loss-of-function mutations of *CLCNKA* has been described. It must be noted that genetic variants of *CLCNKA* have been associated with salt-sensitive hypertension [124]. More recently, a common, loss-of-function variant of *CLCNKA* was identified as a risk factor for Caucasian heart failure [125].

Clinical Manifestations

Patients harboring mutations of ClC-Kb present a broad spectrum of clinical features (Table 2) that range from the aBS/HPS phenotype, characterized by polyhydramnios, isosthenuria, and hypercalciuria, to the classic BS phenotype, with less impaired concentrating ability and normal urinary calcium excretion, to a GS-like phenotype with hypocalciuria and hypomagnesemia [2, 38, 114, 117]. Most patients have episodes of hypokalemic alkalosis and dehydration complicated with muscular hypotonia and lethargy during the first years of life. They are also characterized by increased urinary excretion of PGE2 [2, 38, 114]. The median duration of pregnancy was 38 weeks in the series of Jeck et al. [2], and failure to thrive is common [38, 117]. The diagnosis of Bartter syndrome is usually made during the first year of life, but prenatal (with history of mild maternal polyhydramnios) and late-onset cases are also reported [126]. Most patients with classic BS show failure to thrive and growth retardation [43, 127, 128]. The latter can be due to profound growth hormone deficiency [129]. Like in the antenatal variants, osteopenia with increased markers of bone resorption can be observed [43].

The electrolyte abnormalities are usually severe at presentation, with low plasma Cl^- and severe hypokalemic alkalosis [115]. Increased plasma renin levels, with high or inappropriately normal (with respect to the hypokalemia) aldosterone levels, are typically observed [38, 114]. Polyuria is not uniformly found in classic BS. Iso/-hyposthenuria was only evidenced in approximately one-third of patients, whereas some achieved urinary osmolality above 700 mOsm/kg [2]. The persistence of such a concentrating ability suggests that patients lacking ClC-Kb have a residual TAL function. This is further supported by the fact that only ~20% of patients had sustained hypercalciuria [38]. Nephrocalcinosis was reported in 4/36 affected children [114] but was not detected in three other series [117, 127, 128]. The patients may show a mild hypophosphatemia, which could be related to tubular damage and hypokalemic nephropathy [40, 43]. Isolated cases present with manifestations of renal Fanconi syndrome or distal renal tubular acidosis [117]. About half of the patients lacking ClC-Kb have hypomagnesemia [114]. Several patients harboring mutations in *CLCNKB* show overlapping features of cBS (presentation within the first year of life with episodes of dehydration) and GS (hypomagnesemia with hypocalciuria [114, 119, 130, 131]. Sun et al. [132] reported a patient with cBS who had bilateral sclerochoroidal calcification attributed to persistent hypomagnesemia for 26 years despite magnesium supplementation.

If the full phenotypic spectrum of the Bartter-like syndromes can result from mutations in *CLCNKB*, a significant clinical heterogeneity is observed among patients harboring the same mutation and even between siblings [111, 117, 133]. Mutations in barttin-binding sites of ClC-Kb, dimer interface, or selectivity filter of the channel have been proposed to result in more severe functional consequences [134]. The most common mutation, a homozygous deletion of the entire *CLCKNB*, is typically associated with significant phenotype variability [114, 119, 128, 135]. Yet the authors of a large series of 115 patients reported an association of complete loss-of-function mutations with younger age at diagnosis and CKD [13]. Ethnic differences may participate in the phenotype variability [2]. Indeed, the two original patients described by Bartter were of African American origin [1], and early reports suggested that the course of BS may be more severe in African Americans [136].

A vascular hyporeactivity to the infusion of angiotensin II was originally described by Bartter et al. [1]. This feature is not consistently observed, probably because vascular hyporeactivity improves after correction of volume depletion or treatment with NSAID. Extensive studies (reviewed in [137]) showed that this vascular hyporeactivity could be due to modifications in the angiotensin II-signaling. In turn, these modifications may prevent the release of free radicals – offering increased protection against cardiovascular remodeling in these patients [137]. Stoff et al. [138] evidenced a defect in platelet aggregation in four subjects with Bartter syndrome, but not in other hypokalemic patients. The platelet abnormality was exacerbated by restriction of dietary sodium and lessened by the administration of PG inhibitors. A circulating metabolite of prostacyclin, 6ketoPGE1, may be responsible for the defect [139].

Bartter syndrome is not classically associated with proteinuria. Kidney biopsies consistently show hyperplasia of the juxtaglomerular apparatus, with minimal or no glomerular or tubular abnormalities [1]. However, a few cases of cBS with proteinuria have been reported. Sardani et al. [140] described a 4-year-old African American child with a homozygous deletion in *CLCNKB* and mild mesangial proliferative glomerulonephritis consistent with C1q nephropathy. The follow-up studies of Bettinelli et al. [127] revealed mild-to-moderate glomerular proteinuria in 6/13

ClC-Kb-deficient patients. It was associated with decreased GFR in four patients and microhematuria in two. Kidney biopsy in two patients revealed diffuse or moderate mesangial hypertrophy [127]. In addition, a few cases of clinical BS with unknown genetic defect presented with focal segmental glomerulosclerosis (FSGS) and kidney failure [141, 142]. One of the patients described by Bartter developed CKD, with evidence of advanced nephrosclerosis, interstitial fibrosis, tubular atrophy, and glomerular hyalinization [143]. Causes of kidney failure in BS include complication of salt wasting (including long-standing hypokalemia, hypovolemic episodes, or nephrocalcinosis), chronic activation of the RAAS with ensuing stimulation of TGF-beta and/or TNF-alpha, and toxicity of NSAID [45, 140, 142]. Impaired kidney function was temporally associated with NSAID therapy in two cBS patients, with biopsy-proven interstitial nephritis and resolution after NSAID withdrawal [45]. However, the pathogenic role of NSAID in causing kidney damage in Bartter syndrome has been questioned by the nature and topology of the histological lesions, the fact that kidney lesions were identified in some patients before initiation of drugs, and the lack of progression of tubulointerstitial lesions over more than a decade under NSAID treatment [48, 144]. Of note, kidney cysts have been identified in patients with classic BS [40, 145], potentially linked to K^+ wasting and secondary aldosteronism [146].

Jeck et al. identified the common T481S variant in *CLCNKB*, which showed significantly increased currents when expressed in oocytes [147] and was associated with essential hypertension in a German cohort [148]. The relevance of these findings has been discussed [149], and linkage of the T481S variant to high BP was not confirmed in a Japanese cohort [150].

Differential Diagnosis, Unusual Associations, and Pseudo-Bartter Syndromes

The differential diagnosis of cBS includes the surreptitious use of loop diuretics, laxative abuse [151], which are both unusual in children [152], and chronic vomiting [153]. Measurement of urinary Cl^- and urine screen for diuretics are usually useful to diagnose these patients [154, 155].

The association of hypokalemic metabolic alkalosis with hyperreninemic secondary aldosteronism is also found in other familial disorders affecting the kidneys or the gastrointestinal tract or can be acquired. Generalized dysfunction of the proximal tubule (renal Fanconi syndrome), for instance, due to cystinosis [156], or Kearns-Sayre syndrome, a mitochondrial cytopathy caused by large deletions in mitochondrial DNA leading to cytochrome c oxidase deficiency [157], can be associated with biochemical features resembling BS. A case of familial kidney dysplasia with hypokalemic alkalosis has been reported [158]. Patients with cystic fibrosis are prone to develop episodes of hyponatremic, hypochloremic dehydration with metabolic alkalosis [159, 160]. As mentioned earlier, the Cl^-channel CFTR, which is mutated in cystic fibrosis, may regulate the function of ROMK in the TAL [78]. Gastrointestinal malformations which are associated with Cl^- deficiency [161], or Hirschsprung disease [162], can also lead to pseudo-Bartter syndrome. Administration of prostaglandins in neonates with a ductus-dependent congenital cardiopathy [163], aminoglycosides [164, 165] or combined chemotherapy [166] can induce the biochemical features of BS. Bartter syndrome has also been reported in association with autoimmune diseases, for instance, with Sjögren syndrome [167, 168]. Güllner et al. [169] described a syndrome of familial hypokalemic alkalosis in a sibship presenting with hyperreninemia, aldosteronism, high urinary prostaglandin E2 excretion, normal BP, and resistance to angiotensin II. At variance with BS, the patients had hypouricemia, indicative of proximal tubule dysfunction, and the fractional chloride reabsorption in the TAL was normal. The kidney biopsy showed hypertrophy of tubular basement membranes, whereas the juxtaglomerular apparatus was apparently normal. Finally, the association of BS with a partially empty sella detected by MRI of the brain has been reported in both adult and pediatric patients [170, 171].

Treatment

Patients with cBS are typically treated with PG synthetase inhibitors and escalating doses of KCl, complemented with K^+-sparing diuretics (most often, spironolactone) and NaCl in some of them [48, 127, 128]. Indomethacin is the most frequently used drug, usually started within the first 4 years of life at doses ranging from 1 to 2.5 mg/kg/d. Doses above 3 mg/kg/d are considered nephrotoxic. Indomethacin is well tolerated, but one should remain cautious for signs of gastrointestinal complications [40] or alteration of kidney function [45, 127]. Selective COX2 inhibitors, such as rofecoxib, have been used instead of indomethacin [40] and are currently being evaluated on a larger scale. Potassium supplementation (usually KCl, 1 to 3 mmol/kg/d) is mandatory in cBS, as hypokalemia is often severe at presentation and is not fully corrected by indomethacin [128]. If KCl alone fails to correct hypokalemia, then addition of spironolactone (1 to 1.5 mg/kg/d) is recommended. ACE inhibitors, which have been used for treating hypokalemia in adults with BS [172], should be used with caution given the risk of hypotension. Magnesium supplementation should be added when hypomagnesemia is present, but the correction is typically difficult [38]. Some patients with cBS require gastrostomy tube placement and enteral feeding [128].

The long-term efficacy of the standard treatment with indomethacin and KCl supplementation has been established in cBS. Most biochemical features improve with therapy, although K^+ levels are typically difficult to normalize in most patients despite NSAIDs, KCl, and spironolactone. Treatment also results in improved height and weight, but catch-up growth is inadequate and there is persistent height retardation [40, 48, 127, 128]. Growth hormone deficiency has been demonstrated in some patients, with a positive effect of recombinant human hormone treatment [127, 129]. As discussed above, some cases of cBS are complicated by CKD. A few cases of living-related kidney transplantation have been reported, with improvement of biochemical and hormonal abnormalities after transplantation [141, 144, 173].

Perioperative management of patients with cBS requires a particular care for volume repletion and correction of electrolyte abnormalities during anesthesia and the continuation of anti-prostaglandin therapy to prevent the defective platelet aggregation [174, 175].

Gitelman Syndrome

Genetics

Gitelman syndrome (GS) (MIM #263800) is generally considered as a milder disorder than BS and, with a prevalence of ~1 per 40,000 and potentially higher in Asia, arguably the most frequent inherited tubulopathy detected in adults [176]. The syndrome was first described in 1966 by Gitelman and coworkers as a familial disorder in which patients presented with hypokalemic alkalosis and a peculiar susceptibility to carpopedal spasm and tetany due to hypomagnesemia [177]. For more than 20 years, GS was assimilated with BS. In 1992, Bettinelli and coworkers concluded that GS could be distinguished from BS, based on low urinary Ca^{2+} excretion (molar urinary calcium/creatinine ratio less than or equal to 0.20) [178]. Also, BS patients were more often born after pregnancies complicated by polyhydramnios or premature delivery and had short stature, polyuria, polydipsia, and tendency to dehydration during infancy and childhood, whereas GS patients presented tetanic episodes or short stature at school age [178]. The dissociation of the tubular handling of Ca^{2+} and Mg^{2+} in GS, together with the subnormal response of these patients to thiazides [179, 180], pointed to a primary defect in the DCT.

In 1996, Simon and colleagues demonstrated that presumable loss-of-function mutations in the *SLC12A3* gene were responsible for GS [12]. *SLC12A3* is located on chromosome 16q13 and comprises 26 exons. It encodes the thiazide-sensitive Na^+-Cl^- cotransporter (NCC), a 1021 amino acid integral membrane protein expressed in the apical membrane of cells lining the DCT (Fig. 1). NCC belongs to the SLC12 family of electroneural cation-chloride coupled cotransporters that also includes the Na^+-K^+-$2Cl^-$ and the K^+-Cl^- cotransporters [181]. NCC contains a central hydrophobic region comprising 12 putative transmembrane domains flanked by a short

N-terminal and a long C-terminal hydrophilic intracellular terminus [182]. A model suggests that the affinity-modifying residues for Cl^- are located within TM 1 to 7 and for thiazides between TM 8 to 12 and that both segments are implicated in defining Na^+ affinity of the cotransporter [183]. The activity of NCC is regulated by posttranslational modifications such as glycosylation, phosphorylation, and ubiquitination. Glycosylation sites are found at the connecting loop between TM regions 7–8 (amino acids N406 and N426 in human); NCC is functional in a highly glycosylated homodimeric form. Phosphorylation of NCC is critical for maximal NaCl transport capacity and can alter NCC membrane abundance. Phosphorylation sites are found in the N-termini (amino acids T55, T60, S73, and S91). The phosphorylation of NCC is regulated by a variety of hormonal stimuli, which exert several of their effects via activation of the WNK-SPAK kinase cascade. Finally, NCC is regulated by ubiquitination via the aldosterone-SGK1-Nedd4-2 pathway; Nedd4-2 facilitates the degradation of non-phosphorylated NCC. In addition, it has been recently shown that Nedd4-2 inhibits the activity of the basolateral potassium channels Kir4.1/Kir5.1, which is associated with membrane depolarization and reduction of the driving force for Cl^- exit. The result is an increase of intracellular Cl^- concentration, which is known to inhibit WNK and SPAK. The final result is the inhibition of NCC phosphorylation and activity [183–186].

Gitelman syndrome is transmitted as an autosomal recessive trait, and the majority of patients are compound heterozygous for different mutations in *SLC12A3*. To date, more than 500 mutations scattered through *SLC12A3* have been identified in GS patients (http://www.hgmd.cf.ac.uk). Most (~53%) are missense mutations substituting conserved amino acid residues, whereas nonsense, frameshift, and splice-site defects and gene rearrangements are less frequent. A significant number of GS patients, up to 40% in some series, are found to carry only a single mutation in *SLC12A3*, instead of being compound heterozygous or homozygous [116, 187–189]. Because GS is recessively inherited, it is likely that there is a failure to identify the second mutation in regulatory fragments, 5′ or 3′ untranslated regions, or deeper intronic sequences of *SLC12A3* or that there are large genomic rearrangements. Nozu et al. were the first to report a deep intronic *SLC12A3* mutation in a GS patient, creating a new donor splice site that results in the inclusion of a novel cryptic exon in mRNA [190]. Lo et al. confirmed that deep intronic mutations in *SLC12A3* causing defective expression of NCC can be detected with an RNA-based approach in GS patients [191]. They suggested that analysis of cDNA from blood leukocytes to detect deep intronic mutations should be considered for GS patients who have undetectable or uniallelic *SLC12A3* mutations [191]. Recently, Nozu et al. identify the second variant in another GS patient using next-generation sequencing (NGS) technology; it was a deep intronic mutation creating a new acceptor splice site and inclusion of a cryptic exon [192]. Vargas-Poussou et al. showed that there is a predisposition to large rearrangements caused by the presence of repeated sequences within *SLC12A3*. These large rearrangements, which may account for up to 6% of all *SLC12A3* mutations, can be detected by multiplex ligation-dependent probe amplification (MLPA) or by NGS panels with read depth using adequate bioinformatic tools, enhancing the sensitivity of genetic testing to >80% [14, 116]. As discussed above, mutations in *CLCNKB* have been detected in a few patients presenting simultaneous features of cBS and GS [119, 130, 131] or phenocopying GS. In a cohort of 115 patients with *CLCNKB* pathogenic variants, 26% of cases had a Gitelman-like phenotype [13]. The distribution of ClC-Kb in both the TAL and DCT, and potential compensation by other Cl^- transporters, may probably explain these overlapping syndromes [2]. However, it is possible that other genes participate in the complex handling of Na^+, Ca^{2+}, and Mg^{2+} in DCT or its regulation phenocopying GS [193].

Pathophysiology

The functional effects of mutant NCC were tested using *Xenopus laevis* oocytes and in mammalian cells (HEK293 and MDCKI) [184, 189, 194–197]. Functional analyses revealed that some mutant NCC proteins were synthesized but not properly glycosylated or phosphorylated, targeted for degradation, and not delivered to the plasma membrane [194, 197]. Another class of *SLC12A3* mutations results in normal glycosylated proteins partly impaired in their routing and insertion that perform normal function once they reach the plasma membrane [195, 196]. Other mutants affect the intrinsic activity of the cotransporter, with normal glycosylation and plasma membrane insertion [189]. Finally, splicing mutations of *SLC12A3* result in truncated transcripts that trigger nonsense-mediated decay (NMD), a mRNA surveillance pathway that allows cells to degrade mRNA that contains premature translation stop codons [189]. Interestingly, missense mutation affecting exonic splicing enhancer (ESE) sequences can also cause exon skipping and premature termination [198]. Taken together, these results imply that GS may arise from impaired protein synthesis (splicing mutants); defective processing; defective protein insertion of functional mutants; and defective intrinsic activity of the mutant NCC in the DCT cells [189, 197, 199].

Schultheis and colleagues generated a mouse model with a null mutation in the *Slc12a3* gene on a mixed background [200]. The NCC-null mice showed hypocalciuria and hypomagnesemia at baseline but, in marked contrast to GS patients, no hypokalemic metabolic alkalosis. These mice had no signs of hypovolemia on a standard Na^+ diet, but they showed a lower BP than wild type when fed a Na^+-depleted diet for 2 weeks suggesting a subtle hypovolemia compensated at baseline. Subsequent studies performed on a homogeneous C57BL/6 strain showed that NCC-null mice had a mild compensated alkalosis with increased levels of plasma aldosterone [201] and an increased sensitivity to develop hypokalemia when exposed to dietary K^+ reduction [202]. Belge and coworkers showed that mice lacking parvalbumin, a cytosolic Ca^{2+}-binding protein that is selectively expressed in the DCT, had a phenotype resembling GS, with volume contraction, aldosteronism and K^+ loss at baseline, impaired response to hydrochlorothiazide, and higher bone mineral density [203]. They demonstrated that these modifications were due to modifications in intracellular Ca^{2+} signaling and decreased expression of NCC in the DCT [203].

Studies of inactivating *SLC12A3* mutations and mouse models indicate that the GS phenotype results from dysfunction of NCC. The functional loss of NCC in the DCT leads to salt wasting, volume contraction, stimulation of the RAAS, and increased excretion of K^+ and H^+ in the collecting duct resulting in hypokalemic metabolic alkalosis. By contrast, the pathogenesis of hypocalciuria and hypomagnesemia remains debated. Two hypotheses prevail respecting hypocalciuria. First, the volume contraction causes a compensatory increase in proximal Na^+ reabsorption, driving passive Ca^{2+} transport in the PT [204]. Second, the epithelial cells of the DCT hyperpolarize, due to lower intracellular Cl^- activity, which opens the apical voltage-dependent Ca^{2+} channels (TRPV5), resulting in increased Ca^{2+} influx and reabsorption [205]. Such hyperpolarization could also stimulate the basolateral Na^+/Ca^{2+} exchanger, further increasing Ca^{2+} reabsorption [206]. Studies performed in chronic hydrochlorothiazide-treated mice [207] favor the first hypothesis, as micropuncture experiments demonstrated increased reabsorption of Na^+ and Ca^{2+} in the proximal tubule, whereas Ca^{2+} reabsorption in the distal convolution was unaffected. Furthermore, micropuncture experiments performed in NCC-deficient mice revealed an enhanced fractional reabsorption of Na^+ and Ca^{2+} upstream of the DCT to compensate the transport defect in that segment [201]. The state of the extracellular fluid volume – substantially reduced or not – may also influence the type of tubular mechanism involved [208].

Hypomagnesemia is an essential feature of GS. Several mechanisms, including K^+ depletion, increased passive Mg^{2+} secretion, or defective active Mg^{2+} transport in the DCT, have been proposed to explain the Mg^{2+} wasting [4, 209]. The identification of TRPM6 as a Mg^{2+}-permeable channel in the DCT and its involvement in the pathogenesis of autosomal recessive hypomagnesemia (*see below*) suggested that this channel constitutes the apical entry step in active Mg^{2+} reabsorption [210]. Indeed, chronic thiazide administration increased Mg^{2+} excretion and reduced expression levels of TRPM6 in mice. In addition, TRPM6 expression was also drastically decreased in mice lacking NCC [207]. These results suggest that the pathogenesis of hypomagnesemia in chronic thiazide treatment as well as GS could involve TRPM6 downregulation. Structural variations in the epithelial cells lining DCT, with decreased absorptive surface area for Mg^{2+}, may also play a role [200, 211]. A study of NCC-deficient mice in the first weeks of life suggests that the shortening of the DCT in adult mice is probably the consequence of incomplete outgrowth of DCT1 during early perinatal kidney development [212]; this atrophy correlates with a marked reduction in the abundance of the TRPM6 channel. In addition, it has been suggested that hormonal pathways could co-regulate NCC and TRPM6 and that modifications of basolateral transport of Na and K may contribute to hypomagnesemia [213].

A large body of evidence has emerged that a complex signaling cascade involving a set of kinases (with no lysine kinases, WNK; sterile 20/SPS1-related proline/alanine-rich kinase, SPAK; oxidative stress-responsive kinase-1, OSR1) can activate members of the SCL12A family by phosphorylation of conserved Ser/Thr residues in their N-terminal domain. The importance of this pathway has been confirmed by the manifestations of GS caused by defective NCC and pNCC expression, observed in the SPAK-null mice generated by Yang et al. [214]. Using microarray studies, combined with biochemical, cell biological, and physiological phenotyping, Grimm and colleagues evaluated compensatory pathways in absence of NCC phosphorylation in SPAK-null mice. They showed an adaptatory network to increase salt reabsorption in several nephron segments including upregulation of Na transport in principal cells of connecting tubule and collecting ducts; upregulation of NaCl reabsorption in β-intercalated cells; remodeling of these segments to increase the number of principal and β-intercalated cells; and finally an increase of α-ketoglutarate (α-KG) production by proximal cells, which exercise a paracrine activity to increase NaCl reabsorption in β-intercalated cells [215].

Clinical Manifestations

Classically, GS was considered as a benign variant of Bartter-like syndromes, usually detected during adolescence or adulthood. Since the disorder originates from the DCT, the salt and water losses in GS patients are less pronounced than in aBS or cBS because urinary concentrating ability should not be affected (Table 2). The GS patients are often asymptomatic or present with mild symptoms such as weakness, fatigue, salt craving, thirst, nocturia, constipation, or cramps. They may also consult for growth retardation and short stature, reflecting an alteration in the growth hormone-insulin-like growth factor I axis or pleiotropic effects resulting from magnesium depletion [216]. Typical manifestations include muscle weakness, carpopedal spasms, or tetanic episodes triggered by hypomagnesemia [71, 217]. Blood pressure is reduced, particularly for patients with severe hypokalemia and hypomagnesemia [218]. However, high BP should not rule out the diagnosis, particularly in older patients. Indeed, mild hypertension has been repeatedly reported in GS patients [219, 220]. Since Mg^{2+} ions increase the solubility of calcium pyrophosphate crystals and are important for the activity of pyrophosphatases, hypomagnesemia may promote the formation of calcium pyrophosphate crystals in joints and sclera, leading to chondrocalcinosis [221] and sclerochoroidal calcifications [222]. Patients with GS have higher bone mineral density, similar to chronic thiazide treatment, which likely arises from increased Ca^{2+} reabsorption and a decreased rate of bone remodeling [223]. Potassium and Mg^{2+} depletion can prolong the duration of the action potential in cardiomyocytes and can result in prolonged QT interval, which could lead to an increased risk for ventricular arrhythmias. Prolonged QT interval was initially described in ~50% of the patients [224, 225]. However, in a recent study comparing GS patients with controls and using several ECG evaluations and an accurate method of measurement, the percentage of prolonged QT interval was 9%, although QT intervals in GS patients were longer than in controls [226]. The frequency of prolonged QT was even lower (1/90, 1.1%) in a large cohort of Japanese patients [227]. Cases of GS patient who presented with long runs of ventricular tachycardia [228] or ventricular fibrillation with favorable outcome after cardioversion and continuous supplementation [229] have been reported. In addition, hypokalemic rhabdomyolysis has been reported in several (BS and) GS patients [230–232]. Pregnancies in GS appear to have a favorable outcome, provided continuous K^+ and Mg^{2+} supplementation and monitoring for oligohydramnios [233, 234]. A summary of the manifestations associated with GS and their frequency is shown in Table 3.

Several case reports associating autoimmune disease and genetically confirmed GS have been described [235–240]. In these cases the thyroid is often the affected organ. In a large cohort of Japanese GS patients, the prevalence of thyroid dysfunction was 4.4% [227]. Evans et al. investigated immunity in a group of patients with salt-losing tubulopathies (SLT) including 22 patients with GS [241]. They reported increased bacterial and fungal infections, increased prevalence of eczema/dermatitis and allergic disease, and the presence of autoimmune phenomena in 30% of their SLT cases. Using 23Na-MRI, they demonstrated that sodium stores are reduced in SLT patients compared to controls, which impacts T cell polarization and impairs IL-17 responses, leading to immunodeficiency. This phenotype is comparable with pattern observed in patients with IL-17 deficiency secondary to hereditary anomalies in the transcription factors STAT1/3 (chronic mucocutaneous candidiasis/Hyper-IgE recurrent infection syndrome). Moreover, they confirmed that the addition of NaCl to Th17 lymphocytes rescued polarization and IL-17 production, raising the possibility that correction of extracellular ionic concentrations in SLT patients may mitigate infection risk.

Another clinical manifestation described in GS patients is vertigo. In a French cohort, vertigo without orthostatic hypertension was described in 22% (20/90) of cases [242]. In this study, an adapted questionnaire and complete neuro-ontological exam in 16 cases showed that vertigo was of short duration and triggered in half of patients by head rotation. Seven patients had a vestibular syndrome. Five patients had associated auditory signs (tinnitus, aural fullness, or hearing loss). The expression of NCC in the medial part of the endolymphatic sac in the inner ear [243] and the frequency of vestibular disorder in GS patients (7/90, 8%) suggest a role of NCC in the regulation of endolymphatic composition and/or volume.

The classical biological features of GS include hypokalemic metabolic alkalosis, hypomagnesemia, and hypocalciuria. The recently published consensus [176] proposed the following criteria for suspecting a diagnosis of GS: documented chronic hypokalemia (<3.5 mmol/l) concomitant with inappropriate potassium wasting (spot urine, potassium-creatinine ratio >2.0 mmol/mmol [>18 mmol/g]), in absence of potassium-lowering drugs; metabolic alkalosis; hypomagnesemia (<0.7 mmol/l [<1.70 mg/dl]); inappropriate magnesium wasting 19 (fractional excretion of magnesium >4%); hypocalciuria (spot urine, calcium-creatinine ratio <0.2 mmol/

Table 3 Clinical manifestations associated with Gitelman syndrome

Most common (> 50% of patients)	Prominent (20 to 50% of patients)	Occasional (Less than 20%)	Rare (Case reports)
Salt craving Cramps, muscle weakness, pain Fatigue Dizziness Nocturia Thirst, polydipsia Paresthesia, numbness Palpitations Low blood pressure	Fainting Polyuria Arthralgia Chondrocalcinosis Prolonged corrected QT interval Febrile episodes	Early onset (before age 6) Failure to thrive Growth retardation Vertigo, ataxia Carpopedal spasm, tetany Vomiting Constipation Enuresis Paralysis	Seizure Ventricular tachycardia Rhabdomyolysis Blurred vision Pseudotumor cerebri Sclerochoroidal calcifications

mmol [<0.07 mg/mg]) in adults; calcium-creatinine ratio in children should be interpreted according to age [244]; high renin (activity or plasma levels); fractional excretion of chloride >0.5%; normal or low BP; and normal renal ultrasound with absence of nephrocalcinosis or kidney abnormalities. The presence of both hypomagnesemia and hypocalciuria is highly predictive for the diagnosis of GS [178]. However, there are inter- and intra-individual variations in the extent of hypocalciuria, and hypomagnesemia may be absent in some patients [2, 38, 245]. In the large Japanese cohort described by Fujimura et al., 45% of GS cases showed normal serum magnesium level, and a higher values were observed in GS patients harboring hotspots variants [227]. In addition, the combination of hypocalciuria and hypomagnesemia is also detected in rare cases of cBS patients [130]. Although the urinary PGE_2 excretion is classically normal in GS, increased values of PGE2 or its metabolites can also be detected in some patients with GS [2, 246]. Recently, Vigano et al. showed a hitherto unrecognized tendency toward phosphate wasting with mild to moderate hypophosphatemia that is not related to an altered circulating level of either 25-hydroxyvitamin D or parathyroid hormone [247]. Interestingly, studies in the mouse model of GS showed high FGF23 and PTH levels without modifications of 1,25(OH)2D3, fractional phosphate excretion, and expression of the NaPi2a cotransporter, the FGFR1 receptor, and the co-receptor Klotho. The FGF23 levels were decreased by eplerenone indicating that FGF23 upregulation is aldosterone-mediated [248]. Further studies are necessary to determine whether FGF23 is increased in GS patients with hypophosphatemia.

Several studies described abnormal glucose metabolism and insulin secretion, as well as an increased risk for the development of type 2 diabetes in GS patients, probably due to hypokalemia and hypomagnesemia [249–251]. In the Chinese cohort described by Liu et al., 48% of GS patients had abnormal glucose metabolism and 21% had type 2 diabetes [238]. In the cross-sectional study published by Blanchard et al., GS patients had resistance to insulin potentiated by high BMI, 14% of GS patients having a prediabetes status. The subgroup of GS patients with severe hypomagnesemia had higher insulin resistance index and a tendency toward higher BMI irrespective of the presence of severe hypokalemia, suggesting that magnesium depletion is the predominant contributor to insulin resistance [252]. To decrease risk of diabetes in patients with GS, both low glucose diet and prevention of weight gain are recommended.

No specific findings are observed at kidney biopsy, apart from occasional hypertrophy of the juxta-glomerular apparatus and markedly reduced expression of NCC by immunohistochemistry [253]. Interestingly, a few cases of GS associated with glomerular abnormalities and/or proteinuria have been described. Focal segmental glomerulosclerosis was described in a 21-year-old patient who underwent a biopsy for evaluation of chronic hypokalemia [254]. Hanevold et al. [255] reported a case of focal segmental glomerulosclerosis and C1q nephropathy in an African American child with GS who subsequently developed nephrotic range proteinuria. Another case of focal segmental glomerulosclerosis was reported by Ceri et al. in a 32-year-old man with GS and persistent proteinuria [256]. A retrospective analysis of GS patients revealed proteinuria with preserved kidney function in 6/36 patients. Three of these six patients presented hypertension. No kidney biopsy was performed [220]. Demoulin et al. reported a case of genetically proven GS with glomerular proteinuria and significant abnormalities of the glomerular basement membrane (GBM). Interestingly, defects of the GBM and podocytes were also detected in the *Slc12a3* KO mouse model [257]. These observations point to the possible link between the functional loss of NCC and podocyte dysfunction, potentially due to angiotensin II- or renin-induced podocyte lesions or to chronic hypokalemia [257].

Phenotype Variability and Potential Severity of GS

The view that GS is a benign condition has been challenged by reports emphasizing the phenotype variability and the potential severity of the disease. An evaluation of 50 patients with identified

SLC12A3 mutations revealed that GS is associated with a significant reduction in the quality of life – similar to that associated with congestive heart failure or diabetes [258]. Manifestations such as early onset (before age of 6), growth retardation, invalidating chondrocalcinosis, tetany, rhabdomyolysis, seizures, and ventricular arrhythmia have been described, although in a limited number of cases [38, 189, 216, 228, 258]. Using a standardized questionnaire, quality of life was evaluated in 11 patients with SLT, including 9 GS patients, and the results correlated with biochemical parameters [259]. Higher plasma aldosterone levels were correlated significantly with higher limitations due to physical health; whereas higher magnesium was significantly correlated with better mental health.

Chronic hyperaldosteronism seems to not have effect on cardiac function in GS patients. A recent study analyzed global left ventricular mass index and intracellular and extracellular mass index using cardiac magnetic resonance in patients with different etiologies of hyperaldosteronism [260]. Four groups of 20 individuals with primary aldosteronism, essential hypertension, GS, and healthy control were compared. Only primary aldosteronism combined with high BP increased both extracellular myocardial matrix and intracellular mass. Secondary aldosterone excess with normal BP, i.e., the situation in GS patients, did not affect extracellular myocardial matrix, a measure of myocardial fibrosis in vivo.

The phenotype of GS is highly heterogeneous in terms of age at presentation, gender, biochemical abnormalities, and clinical manifestations (Table 3). The phenotype variability has been documented not only between patients carrying different *SLC12A3* mutations but also for a common underlying mutation [261] and between affected family members [189, 262]. In Chinese cohorts, females have a less severe phenotype than males [238, 234, 246]. The mechanisms that could account for intrafamilial variability include gender (affected brothers are apparently more severely affected than their sisters carrying the same mutation), modifier genes (affecting the regulation or activity of NCC), and environmental factors (dietary intake of NaCl, Ca^{2+}, or Mg^{2+}) [2, 189, 262, 263]. Compensatory mechanisms operating in other nephron segments should also be considered, as evidenced in mice with defective NCC [201, 203, 207]. Considering that most of patients with GS are compound heterozygous harboring various mutant *SLC12A3* alleles, the phenotype variability could be related to the nature and/or position of the underlying mutation(s). This hypothesis has been substantiated by the studies of Riveira-Munoz et al. [189] which showed that a specific combination of mutations was preferentially associated with a severe presentation of GS. Balavoine et al. showed a more severe phenotype in GS patients with two mutated alleles than in those with one or no mutated alleles [219]. A follow-up study in a large cohort of 117 Taiwanese GS patients demonstrated that male patients had an earlier age of onset, more severe hypokalemia, and significantly lower serum aldosterone concentration and that patients with homozygous and deep mutations in intron 13 had a more severe phenotype [250]. Conversely, Berry et al. reported an apparently more severe electrolyte disturbance in women, which could possibly be due to the effects of female hormones on expression or function of NCC [220].

Blood Pressure in GS: Effect of the Carrier State

In 2001, Cruz et al. investigated a large Amish kindred to show that patients with GS had significantly lower age- and gender-adjusted diastolic and systolic BP, a higher urinary Na^+ excretion, and a higher salt intake than their unaffected relatives [218]. Additional support for the role of NCC in BP regulation was provided by the report that transplantation of a GS kidney into a non-Gitelman hypertensive recipient resulted in the correction of hypertension in the latter [264]. Considering that the frequency of heterozygote carriers of *SLC12A3* mutations varies from 1% to 4% [265, 252], the question was thus raised whether single loss-of-function mutations in *SLC12A3* may affect BP regulation in the general population [54]. Lifton and colleagues screened

3125 adult subjects from the Framingham Heart Study for mutations in *SLC12A3* (and by extension *SLC12A1*, and *KCNJ1*, responsible for aBS) and identified 30 different mutations in *SLC12A3*, 10 in *SLC12A1*, and 5 in *KCNJ1*) in 49 subjects [52]. Of these mutations, 10 were biochemically proven loss of function (7 in NCC alone), and 20 were inferred from the conservation and rarity criteria. Examination of long-term BP revealed that 80% of the mutation carriers were below the mean systolic BP values of the entire cohort. The mean BP reduction in carriers was similar to values obtained with chronic thiazide treatment [52]. This study was replicated in a population of 7444 European ancestry individuals from the Atherosclerosis Risk in Communities study [266].

The studies described above analyzed a small number of individuals from very large unselected cohorts using office BP evaluation. To better evaluate the phenotype of heterozygous carriers of *SLC12A3* mutations, Blanchard et al. performed a cross-sectional study comparing 81 heterozygous carriers, 82 healthy noncarriers, and 79 patients with GS of similar age, body mass index, and sex ratio [252]. In this study the heterozygous patients were relatives of GS patients, BP was evaluated by home monitoring, and biochemical analyses included oral glucose tolerance testing and assays of plasma and urine electrolyte and hormone levels. They failed to confirm a protective effect against hypertension in heterozygous carriers although a subtle intermediate phenotype, characterized by a slightly increased plasma aldosterone, slightly higher plasma calcium, and lower plasma PTH concentrations compatible with an increase in reabsorption of calcium, was observed. Thus, larger studies are required to confirm a phenotype associated with the carrier state of GS.

Differential Diagnosis

The differential diagnosis of GS includes other Bartter-like syndromes (Tables 1 and 2) and particularly cBS due to mutations in *CLCNKB* [2, 245], as well as diuretic or laxative abuse, and chronic vomiting. As mentioned above, the clinical history and biochemical features, even hypocalciuria and hypomagnesemia, may not be fully reliable to distinguish GS from cBS. In the clinical setting, the evaluation of the response to hydrochlorothiazide (HCTZ test) offers a high sensitivity and specificity for the diagnosis of GS [245, 267]. However, in view of the rapid progress of genetic testing, HCTZ test is no longer recommended as a diagnostic tool in GS because of the related risks: when it is used diagnostically there is a risk of acute volume depletion in subjects with loop of Henle defect [268, 176]

Patients with molecular anomalies in the *HNF1B* gene coding for transcription factor HNF1β can mimic GS. Hypomagnesemia, hypokalemia, and hypocalciuria can be present in *HNF1B*-related disorders [269, 270]. In the recent study in the UK cohort, hypomagnesemia was constant when evaluated in the second decade of life [271]. HNF1β has been shown to regulate expression of the γ-subunit of the basolateral Na^+-K^+-ATPase (FXYD2) and the basolateral potassium channel kir5.1, which in turn regulates the activity of the Na^+-K^+-ATPase, which is crucial for the transport activity in the DCT [272, 273]. The presence of a family history, dominant mode of inheritance, maturity onset diabetes of the young, chronic kidney disease, and other renal and extrarenal anomalies described in *HNF1B*-related disorders can help to orientate the diagnosis [274].

Patients with pathogenic variants in the *FXYD2* gene were described as a cause of isolated hypomagnesemia; however, subsequent data on newly discovered patients also show a trend to a Gitelman-like tubulopathy [275]. This is in agreement with the known pathophysiology of HNF1β as transcription factor.

Mutations in the *KCNJ10* gene coding for the inwardly rectifying potassium channel Kir4.1 cause EAST syndrome, an autosomal recessive disorder characterized by epilepsy, ataxia, sensorineural deafness, and tubulopathy with the same characteristics of GS [276, 277]. Kir4.1 forms heterodimers with Kir5.1 channel, which regulate the activity of the basolateral Na^+-K^+-ATPase. The extrarenal features of EAST syndrome allow it to be distinguished from GS.

Nowadays, the use of high-throughput sequencing is recommended for diagnosis of GS [176]. It allows the analysis at the same time of genes phenocopying GS included in specific panels [14, 278] or in the future the analysis of in silico panels after whole exome or whole genome sequencing.

Gitelman syndrome-like manifestations including hypokalemic metabolic alkalosis with hypomagnesemia and hypocalciuria have been reported as a rare complication of the use of cisplatin [279]. Although the mechanism remains uncertain, cisplatin is known to induce focal tubular necrosis lesions in the DCT [280]. Autoimmune disorders cause acquired kidney tubular disorders, potentially due to autoantibodies against tubular components [281]. Typical features of acquired GS have been reported in association with various autoimmune disorders including iritis and arthritis [282], sialoadenitis [168], and Sjögren syndrome [283]. Of note, there was no improvement of K^+ wasting after corticosteroid treatment in one such case [283].

Treatment

Because GS is caused by a primary defect in a NaCl cotransporter, ad libitum NaCl intake should be strongly advocated. It is recommended to encourage patients to follow their propensity for salt consumption ("salt craving") [176]. Lifelong oral magnesium and potassium supplementations are the main treatment in patients with GS. A reasonable target for potassium may be 3.0 mmol/l and magnesium 0.6 mmol/l (1.46 mg/dl) [176]. Magnesium supplementation should be considered first, since Mg^{2+} repletion will facilitate K^+ repletion and reduce the risk of tetany and other complications related to hypomagnesemia [217, 220, 284]. All types of magnesium salts are effective, but their bioavailability is variable. Magnesium chloride, magnesium lactate, and magnesium aspartate show higher bioavailability [217]. $MgCl_2$ is recommended since it will also correct the urinary loss of Cl^-. The dose of magnesium must be adjusted individually in three to four daily administrations, with diarrhea being the limiting factor. The recommended starting dose is 300 mg/day (12.24 mmol) of elemental magnesium (5 mg/kg in children, i.e., 0.2 mmol/kg), in slow-release tablets when possible. In addition to magnesium, high doses of oral KCl supplements may be required; starting doses could be ≥40 mmol KCl (1–2 mmol/kg in children), in divided doses throughout the day [285]. Importantly, Mg^{2+} and K^+ supplementation results in a catch-up growth [189, 216]. Spironolactone or amiloride can be useful, both to increase serum K^+ levels in patients resistant to KCl supplements and to treat Mg^{2+} depletion that is worsened by elevated aldosterone levels [286]. Both drugs should be started cautiously to avoid hypotension. Patients should not be refrained from their usual salt craving, particularly if they practice a regular physical activity. More recently, the potassium-sparing diuretic and aldosterone antagonist eplerenone was shown to be useful in the treatment of GS patients [287–290]. Eplerenone is a selective aldosterone antagonist, with significantly lower affinity for androgen, progesterone, and glucocorticoid receptors in comparison with spironolactone and has therefore no anti-androgenic side effects (such as gynecomastia, hirsutism, erectile dysfunction, and menstrual irregularities) [287]. The usefulness of eplerenone was initially demonstrated in patients with cardiovascular disease and heart failure [291]. Prostaglandin inhibitors such as indomethacin are less indicated in GS than in aBS, since urinary PGE_2 levels are usually normal. Liaw et al. reported an improvement in growth response following high-dose indometacin but complicated by gastrointestinal hemorrhage [292]. Refractory hypokalemia has also been treated with the specific COX-2 inhibitor rofecoxib [293]. Considering the occurrence of prolonged QT interval in GS patients [224, 225], QT-prolonging medications should be used with caution. An open-label, randomized, crossover study was conducted to compare the efficacy and safety of 6-week treatment with one time daily 75 mg slow-release indomethacin, 150 mg eplerenone, or 20 mg amiloride added to constant potassium and magnesium supplementation in 30 GS patients [290]. Each drug increased plasma potassium concentration.

Indomethacin was the most effective, but it was associated with decreased eGFR, and it can cause gastrointestinal intolerance. Amiloride and eplerenone had similar but lower efficacies and increased sodium depletion [290].

Caution should be taken when patients with GS undergo anesthesia because the effects of local and general anesthetic agents can be potentiated by hypokalemia and hypomagnesemia. Although exact and safe preoperative levels of potassium and magnesium have not been established, the UK National Institute for Health and Care Excellence (NICE)-approved guidelines suggest aiming for potassium levels of ≥3.0 mmol/l and magnesium 0.5 mmol/l (1.22 mg/dl) [294].

Although GS adversely affects the quality of life [258], we lack information about the long-term outcome of these patients. Kidney function and growth appear to be normal, provided lifelong supplementation [295]. Progression to kidney failure is rare in GS: only two patients with GS who developed end-stage kidney disease have been reported [296, 297]. However, in the follow-up study of a large cohort of 117 Taiwanese patients, 7/117 (6%) patients developed CKD (stage III-IV) and 5/117 (4.3%) patients developed type 2 diabetes at follow-up (mean duration, 6.7 ± 3.4 years; range, 1–33 years [250]. In addition, despite the salt wasting, development of secondary hypertension may be expected in the aging GS population [220]. In view of recent results, both low glucose diet and prevention of weight gain are recommended in patients with GS to decrease risk of diabetes [176].

Pseudohypoaldosteronism Type I

Type 1 pseudohypoaldosteronism (PHA1) is a rare form of mineralocorticoid resistance characterized by neonatal kidney salt wasting, failure to thrive, and dehydration. It is associated with hyponatremia, hyperkalemia, and metabolic acidosis, despite extremely high values of plasma renin and aldosterone [298]. It was first reported by Cheek and Perry, who described a male infant with severe salt wasting in the absence of any renal or adrenal defect [299]. There exist two different clinical forms of PHA1: (i) a dominantly inherited form, in which mineralocorticoid resistance is restricted to the kidney, and (ii) a recessively inherited form, where mineralocorticoid resistance is systemic and salt loss occurs in multiple organs [300].

Clinical and Biochemical Features

Autosomal dominant PHA1 (MIM#177735, or PHA1A) is a mild form and represents the most frequent form of the disease [301]. The prevalence, as estimated from recruitment through a national reference center for rare diseases (MARHEA, http://www.soc-nephrologie.org/marhea/) is ~1 per 80,000 newborns. Clinical expression is variable: in general, patients show a salt-losing syndrome in the neonatal period, with weight loss, failure to thrive, vomiting, and dehydration. Occasionally, patients may also present hypercalciuria. Biological findings are hyponatremia, hyperkalemia, and inappropriately high urinary sodium excretion. In contrast, urinary potassium excretion is low, with reduced fractional potassium excretion and transtubular potassium gradient [302, 303]. The diagnosis is confirmed by the presence of high plasma and urinary aldosterone and high plasma renin levels. Symptoms of PHA1A usually improve in early childhood. The mechanisms which restore sodium homeostasis in these patients are not clear; most likely, kidney maturation, access to dietary salt, compensatory increase in proximal sodium reabsorption, as well as the upregulation of the mineralocorticoid axis all play a role to compensate for the distal salt loss. A recent case-control study, comparing 39 adult patients with PHA1A carriers of *NR3C2* mutations with sex- and age-paired noncarriers, showed that adult PHA1A subjects present lifelong increased plasma renin and aldosterone levels together with increased salt appetite, with normal potassium levels and BP [304].

Autosomal recessive PHA1 (MIM #264350, or PHA1B), is characterized by severe salt wasting from the kidney, colon, sweat, and salivary glands

[305]. Patients present in the neonatal period with severe dehydration, vomiting, and failure to thrive; acute polyhydramnios has been described [306] but seems exceptional. The clinical picture may be complicated by cardiac dysrhythmias, collapse, shock, or cardiac arrest [307]. Severe hyperkalemia and high aldosterone and plasma renin levels orient the diagnosis that can be completed by a positive salivary or sweat test. Consistent with the systemic mineralocorticoid unresponsiveness, patients present elevated sweat and salivary Na^+ and Cl^- and absent nasal amiloride-sensitive Na^+ transport [300]. In addition to the kidney phenotype, frequent respiratory tract illnesses have been observed, characterized by persistent rhinorrhea of clear liquid, congestion, tachypnea, wheezing, frequent fever, and recurrent pulmonary infections, evoking cystic fibrosis, due to their reduced capacity to absorb liquid from airway surfaces [308]. Also, the high concentration of sweat salt may cause inflammation and damage in the eccrine structures, with cutaneous lesions similar to those appearing in miliaria rubra [307, 309]. PHA1B is more severe than the dominant form (PHA1A), and the prognosis is poor: no remission has been reported and patients suffer from recurrent life-threatening episodes of salt loss. Early diagnosis within the first week of life is critical to survival in generalized PHA1B [310].

Secondary PHA1. Transient pseudohypoaldosteronism has been observed in infants less than 7 months of age suffering from urinary tract malformations or urinary tract infections or both [311, 312]. The incidence has been estimated around 1 per 13,200 total live births per year [313]. Any kind of congenital urinary tract obstruction (ureterohydronephrosis, ureterocele, ureteropelvic junction obstruction, posterior urethral valves) may lead to secondary PHA1. Physiopathological mechanisms responsible for transient PHA1 are poorly understood, but the gravity of the salt wasting is inversely correlated with age, supporting a role for tubular immaturity [310]. In these patients, medical or surgical cure of the primary disease reestablishes the normal response to aldosterone although high salt intake is recommended in the acute phase with progressive tapering. The possibility that a transient tubular mineralocorticoid resistance can arise in infants with urinary tract malformations or urinary tract infections strongly supports an indication for renal ultrasonography and urine cultures in all children presenting with salt wasting and hyperkalemia [311]. Secondary PHA1 may also develop in adults following resection of the ileum and colon [314] or in patients with kidney transplantation treated with immunosuppressants (tacrolimus, cyclosporine), probably due to impairment of the mineralocorticoid receptor transcriptional activity [315].

Pathophysiology and Genetics

Aldosterone plays a key role in electrolyte balance and BP regulation. It confers the main hormonal regulation of sodium, potassium, and hydrogen balance in the aldosterone-sensitive distal nephron through its interaction with the mineralocorticoid receptor [316] (Fig. 3). The MR belongs to the nuclear receptor superfamily and acts as a ligand-activated transcription factor regulating expression of a coordinate set of genes ultimately eliciting physiologic aldosterone responses [317]. In the collecting duct, aldosterone stimulates activity of several proteins implicated in transepithelial Na^+ transport, including ENaC and Na^+-K^+-ATPase [318].

Since the first description of PHA1, a defect of the tubular response to aldosterone had been suggested as the underlying cause. After the characterization of aldosterone binding sites in mononuclear leukocytes, Armanini et al. confirmed this hypothesis by showing that ^3H-aldosterone binding was absent or very low in mononuclear leukocytes of affected patients [319]. In a subsequent study of eight families, a dual pattern of inheritance was observed that correlated with receptor binding abnormalities. In some families, an autosomal recessive inheritance of the disease was observed, and binding studies and aldosterone levels were normal in both parents. In contrast, some families presented with an autosomal dominant inheritance, and low receptor number and elevated plasma aldosterone were always found in one of the parents [320]. The main differences between the dominant (PHA1A) and recessive

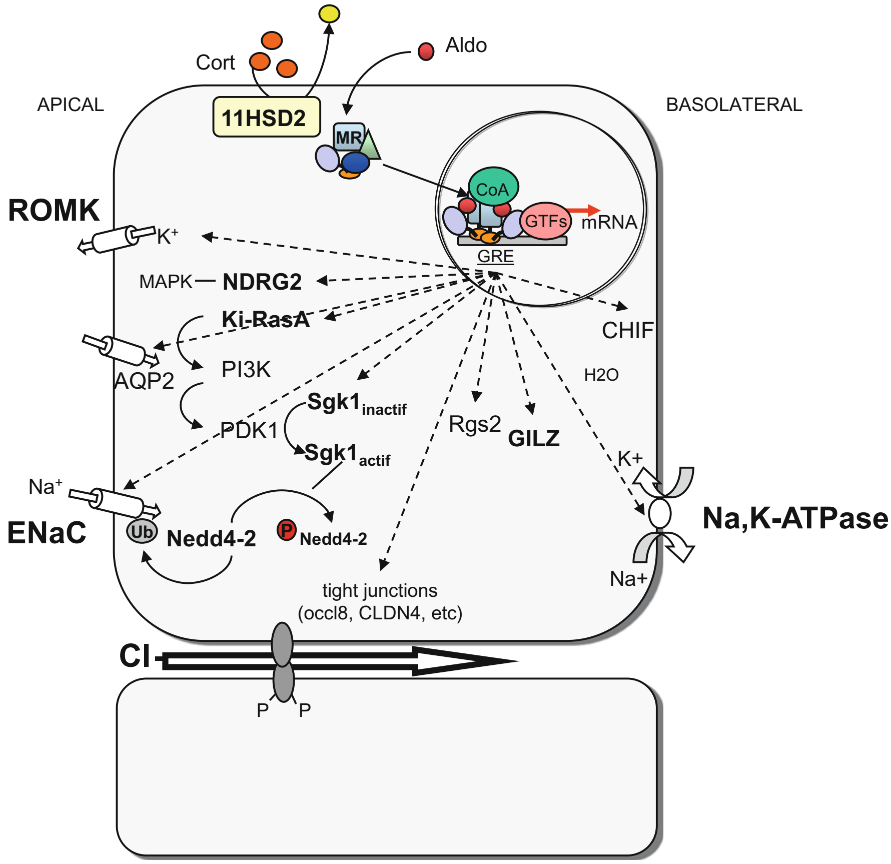
Cort
Aldo
APICAL
BASOLATERAL
11HSD2
MR
CoA
GTFs
mRNA
GRE
ROMK
K+
MAPK
NDRG2
Ki-RasA
AQP2
PI3K
PDK1
Sgk1 inactif
Sgk1 actif
CHIF
H2O
Rgs2
GILZ
K+
Na,K-ATPase
Na+
Na+
ENaC
Ub
Nedd4-2
P
Nedd4-2
tight junctions
(occl8, CLDN4, etc)
Cl-
P
P

Fig. 3 Molecular basis of pseudohypoaldosteronism type I. Aldosterone binds to its intracellular receptor, the mineralocorticoid receptor (MR). Hormone binding induces dissociation of MR-associated proteins and translocation of the aldosterone-receptor complex into the nucleus, where it binds to specific DNA sequences in regulatory regions of hormone-responsive genes. These genes code for proteins involved in transepithelial sodium transport (the epithelial sodium channel, ENaC; the sodium-potassium pump Na,K-ATPase), for regulatory proteins (serum- and glucocorticoid-regulated kinase 1 (sgk1), channel-inducing factor (CHIF), the proto-oncogene K-Ras2, N-myc downregulated gene 2 (NDRG2)) and others. Apical and basolateral indicate the two poles of the epithelial cell. *Abbreviations*. Aldo: aldosterone

(PHA1B) forms of PHA1 are summarized in Table 4.

Subsequently it was shown that the two clinical forms of PHA1 were caused by different genetic defects. Inactivating mutations in the *NR3C2* gene coding for the MR are found in PHA1A [321–323]. The human MR coding gene *NR3C2* spans over ~450 kB on chromosome 4p31.23 and is composed of ten exons [324]. Patients are heterozygous carriers of genetic variants which can be found in both patients with autosomal dominant PHA1A and patients with a sporadic renal presentation [323]. In the latter group, only one-third are *de novo* mutations, implying that carriers develop clinically evident disease only in a small proportion of kindreds. Although the exact causes for the variable phenotypic expression of PHA1A are unknown, intercurrent events, such as infections, vomiting, and diarrhea, may precipitate neonatal salt loss and dehydration in individuals at risk. Also, naturally occurring hypomorphic or hyperfunctioning alleles of other genes, coding for proteins involved in distal sodium reabsorption, may aggravate or attenuate the phenotype.

Table 4 Distinctive clinical and biological features of renal and generalized PHA1

Feature	Autosomal dominant PHA1 (PHA1A)	Autosomal recessive PHA1 (PHA1B)
OMIM	#177735	#264350
Age of onset	Neonatal	Neonatal
Phenotype	Mild	Severe
Failure to thrive	Present	Present
Vomiting	Present	Present
Dehydration episodes	Mild	Severe
Cardiac dysrhythmias, collapse, shock, or cardiac arrest	No	Sometimes
Respiratory tract illnesses	No	Frequent
Eczematoid rash of the skin	No	Sometimes
Hyperkalemic metabolic acidosis	Present	Present
Serum Na^+	↓	↓
Serum K^+	↑	↑
Urinary Na^+ excretion	↑	↑
Urinary Ka^+ excretion	↓	↓
Plasma aldosterone	↑	↑
Plasma renin	↑	↑
Urinary aldosterone	↑	↑
Sweat Na^+	N	↑
Salivary Na^+	N	↑
Type of mutation	Loss of function	Loss-of-function
Gene locus	4q31.1	12p13, 16p13-p12, 16p13-p12
Genes	*NR3C2*	*SCNN1A*, *SCNN1B*, *SCNN1G*
Proteins	MR, mineralocorticoid receptor	α-, β-, γ-subunits of the epithelial Na^+ channel ENaC

After the first description of *NR3C2* mutations in four dominant and one sporadic case of PHA1A [321], more than 100 different causal genetic variants have been reported in patients with PHA1A [325]. Disease-causing variants are located in all exons of the *NR3C2* gene, affecting all functional domains of the MR protein. However, only frameshift, splice site, and nonsense mutations were reported in exon 2, suggesting that missense mutations affecting the N-terminal domain do not sufficiently affect MR function to become clinically apparent.

The pathogenic mechanism of PHA1 in patients with heterozygous MR mutations depends on the mutation. Although haploinsufficiency is sufficient to cause autosomal dominant PHA1A [322, 326], mutated receptors may also exert dominant negative effects on the wild-type receptor since MR regulate transcription by binding as receptor dimer to regulatory regions of target genes [322]. In this case, effects of mutated receptors are strongly promoter-dependent and may differentially affect MR function in a gene-specific manner [327, 328]. Furthermore, the same mutation may induce complete functional loss of transcriptional activity on one promoter while retaining a partial transcriptional activity on another gene [328]. Thus, not only the extent of functional reduction but also the specific qualitative loss of function, in terms of regulated gene expression, modulate the phenotype in PHA1 patients with *NR3C2* mutations.

The severe and generalized, recessive form of PHA1 (PHA1B) is caused by loss-of-function mutations of the genes coding for the subunits of the amiloride-sensitive sodium channel ENaC (*SCNN1A*; *SCNN1B*; *SCNN1G*). ENaC is a hetero-multimeric protein composed of three subunits, α, β, and γ, which form the channels expressed on the cell surface (see review by

479 [329]. The α-subunit of ENaC is encoded by *SCNN1A* located on chromosome 12p13.3, whereas *SCNN1B* (encoding βENaC) and *SCNN1G* (encoding γENaC) are located within 400 kb on chromosome 16p12.2-p13.11. Loss-of-function mutations have been found in affected patients being either homozygous – in consanguineous families – or composite heterozygous [330, 331]. They include missense and nonsense mutations, deletions, insertions, and splice site junction mutations leading to abnormal mRNA splicing. PHA1B-causing variants appear in all ENaC subunits but are more frequent in the α-subunit, consistent with its determinant role in channel function. Missense mutations are found in critically important domains of the protein and affect functions like intracellular trafficking of the channel, channel gating, or the ion-selectivity filter [332].

Genotype-phenotype correlations are not well established in PHA1B. One study has explored the long-term consequences of different αENaC mutations on the renin-aldosterone system, growth, and pubertal development of PHA1B patients [333]. Three patients homozygous for nonsense and frameshift mutations in αENaC presented short stature, poor growth, and growth hormone tests compatible with the diagnosis of GH deficiency. In all patients, there was an age-dependent normalization in the urinary Na/K ratios accompanied by an exaggerated renin-aldosterone system response probably contributing to age-dependent amelioration. In contrast, one patient compound heterozygous for a missense mutation and for a frameshift mutation of αENaC presented normal growth, normal puberty, and decrease of renin-aldosterone axis activity with age. These results demonstrate distinct genotype-phenotype relationships in PHA1B patients that depend on the degree of functional ENaC impairment. If undetected during the first week of life, multi-system PHA1B can lead to neonatal death; however, if detected, the patients may lead near normal lives on a lifelong high-salt diet [334].

Extensive genetic investigation of PHA1 patients in recent years has also shown that the disease comprises a continuum of phenotypically and/or biologically distinct entities that may challenge the current genetic classification of the disease. Indeed, investigation of heterozygote carriers of a αENaC p.Ser562Pro mutation, responsible for PHA1B in homozygous patients, revealed a subclinical salt-losing phenotype with increased sweat sodium and chloride concentrations without additional hormonal or clinical manifestation [335]. Furthermore, recessively inherited ENaC mutations, associated with partial loss of channel function, may result in a mild phenotypic expression of PHA1B, with a salt-losing disorder in a premature infant, but only a biological phenotype in a sibling born at term [336]. We also observed a case of recessive PHA1A with an extremely severe phenotype of dehydration and hyperkalemia, but without cutaneous or pulmonary phenotype, caused by MR mutations [337]. These cases broaden the spectrum of clinical phenotypes in PHA1A and support corresponding genetic screening for the disease in patients with isolated renal salt-losing syndromes and/or failure to thrive.

Treatment and Prognosis

Treatment of PHA1 consists in the replacement of salt loss and rehydration, as well as correction of hyperkalemia and acidosis in the acute phase of the disease. Since the main differential diagnosis is congenital adrenal hyperplasia or isolated deficiency in aldosterone synthase (CMOI and CMOII) [338], replacement therapy with fludrocortisone and hydrocortisone may be undertaken while confirming the diagnosis by hormonal measurements. Early postnatal hyperkalemia may sometimes complicate antenatal Bartter's syndrome (aBS, due to mutation in the potassium channel ROMK) [51]. Its association with hyponatremia and hyperreninemic hyperaldosteronism may erroneously suggest the diagnosis of PHA1. However, hyperkalemia appears usually very early and normalizes by the end of the first postnatal week, whereas secondary PHA1 is characterized by permanent hyperkalemia. Other distinctive features of aBS patients are metabolic alkalosis as well as hypercalciuria and nephrocalcinosis. Also maternal hydramnios, present in aBS, is a rare event in generalized PHA1.

After the acute period, treatment consists in salt supplementation. The doses vary depending on the severity of the disease. Neonatal genetic diagnosis on cord blood may allow rapid diagnosis and management of the condition in affected offspring from PHA1 families with identified mutations. In PHA1A and secondary PHA1, 3–20 mEq/kg/d of Na+ given as NaCl and NaHCO3- are sufficient to compensate for the salt loss and are followed by rapid clinical and biochemical improvement. The expansion of extracellular volume results in increased tubular flow and delivery of sodium to the distal nephron, creating a favorable gradient for potassium secretion. Nevertheless, ion exchange resins are often associated with the treatment to normalize potassium levels. The amount of sodium required depends on the severity of the symptoms and is deduced from the normalization of plasma potassium concentration and plasma renin. Since PHA1A improves with age, treatment can be discontinued after a variable period of time in most patients, generally around age 18–24 months. Older children are generally asymptomatic on a normal salt intake and show a normal growth and psychomotor development, although they may evolve on the lower percentiles of the growth curve, despite adequate medical therapy [339]. Follow-up of an extended family over four generations over four decades in symptomatic and asymptomatic affected family members showed age-dependent decrease in both plasma renin activity and serum aldosterone levels over the years [340], although both plasma renin and aldosterone levels remain high in adult patients [304]. Early diagnosis in affected family members mitigates the clinical course of the disease. No adverse cardiovascular outcome was reported in adult PHA1A carriers of *NR3C2* mutations, who rather show an improved diastolic left ventricular function [304].

Despite extremely elevated serum aldosterone levels during pregnancy, a recent study suggests that pregnant women may not experience ill effects [340], although careful follow-up is recommended. A higher frequency of miscarriage has been reported in women carrying *NR3C2* mutations [304].

In contrast to PHA1A, generalized PHA1B represents a therapeutic challenge. No evidence-based treatment has been described, and therapeutic intervention is patient-specific. Generally, high doses of sodium (between 20 and 50 mEq/kg/d) are used, together with ion exchange resins and dietary manipulations to reduce potassium levels. Corticoid treatment is sometimes associated and seems to provide some additional benefit. Administration of indomethacin may be useful in occasional patients [341]. Symptomatic treatment is necessary for the respiratory tract illnesses and to correct the skin phenotype. Only few cases of generalized PHA1B followed up for several years or into adulthood have been described: lifelong treatment is necessary, consisting of salt supplementation (8–20 g NaCl/day) and ion exchange resins [310, 334]. A recent study has suggested that dietary potassium restriction may be useful to attenuate urinary sodium wasting in PHA1B, although this result warrants confirmation [341].

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Inherited Disorders of Renal Magnesium Handling

42

Martin Konrad and Karl Peter Schlingmann

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Abstract

Magnesium (Mg^{2+}) depletion usually occurs secondary to another disease process or to a therapeutic agent. During infancy and childhood, a substantial proportion of patients receiving medical attention for signs of hypomagnesemia is affected by inherited renal disorders associated with Mg^{2+} wasting. In these disorders, hypomagnesemia either may be a leading symptom or may be part of a complex phenotype resulting from tubular dysfunction, as detailed below. Advances in molecular genetics of hereditary hypomagnesemia

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substantiated the role of a variety of genes and their encoded proteins in human epithelial Mg^{2+} transport, and helped to characterize different clinical subtypes of hereditary Mg^{2+}-wasting. A careful clinical and biochemical assessment allows to distinguish the different disease entities in most cases, even when there is a considerable overlap in the phenotypic characteristics.

Keywords

Magnesium · Hypomagnesemia · Tubulopathy

Introduction/Physiology

Magnesium (Mg^{2+}) is an important intracellular cation. As a cofactor, it is involved in energy metabolism and protein and nucleic acid synthesis. It is also critical for the modulation of membrane transporters and in signal transduction. Under physiologic conditions, serum Mg^{2+} levels are maintained at almost constant values. Mg^{2+} homeostasis depends on a balanced intestinal absorption and renal excretion. Mg^{2+} deficiency can result from reduced dietary intake, intestinal malabsorption, or renal loss. The control of body Mg^{2+} homeostasis primarily resides in the kidney tubules.

The dietary intake of Mg^{2+} may vary substantially. The principal site of Mg^{2+} absorption is the small intestine, where Mg^{2+} absorption occurs via two different pathways: a saturable active transcellular transport and a nonsaturable paracellular passive transport [1, 2]. In the kidney, approximately 80% of total serum Mg^{2+} is filtered in the glomeruli, of which more than 95% is reabsorbed along the nephron. Tubular Mg^{2+} reabsorption differs in quantity and kinetics depending on the different nephron segments. In adults, approximately 15–20% is reabsorbed in the proximal tubule, whereas the premature kidney of the newborn is able to reabsorb up to 70% of the filtered Mg^{2+} in this nephron segment [3]. From early childhood on, roughly 70% of Mg^{2+} is reabsorbed in the cortical thick ascending limb (TAL) of the loop of Henle. Transport in this segment is passive and paracellular, mediated by claudin-16 and claudin-19. The driving force for reabsorption against an unfavorable concentration gradient is the lumen-positive transepithelial voltage (Fig. 1a). Only 5–10% of the filtered Mg^{2+} is reabsorbed in the distal convoluted tubule (DCT). However, in this part of the nephron the fine adjustment of renal excretion is accomplished. In the DCT, Mg^{2+} transport is an active transcellular process (Fig. 1b). Apical entry is mediated by the specific and regulated Mg^{2+} channel TRPM6. The mechanism of basolateral transport into the interstitium is unknown. Here, Mg^{2+} has to be extruded against an unfavorable electrochemical gradient. Most physiologic studies favor a Na^+-dependent exchange mechanism [4]. Mg^{2+} entry into DCT cells appears to be the rate-limiting step and the site of regulation. In the collecting duct, there is no significant Mg^{2+} uptake. Finally, 3–5% of the filtered Mg^{2+} is excreted in the urine.

Advances in molecular genetics of hereditary hypomagnesemia substantiated the role of a variety of genes and their encoded proteins in human epithelial Mg^{2+} transport, and helped to characterize different clinical subtypes of hereditary Mg^{2+}-wasting (Table 1). A careful clinical and biochemical assessment allows to distinguish the different disease entities in most cases, even when there is a considerable overlap in the phenotypic characteristics (Table 2).

Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive disorder caused by mutations in two different members of the claudin gene family, namely, claudin-16 and claudin-19 [5, 6]. Since its first description, more than 150 patients have been reported allowing a comprehensive characterization of the clinical spectrum [7–17]. As a consequence of excessive renal Mg^{2+} and Ca^{2+} wasting, patients develop the characteristic triad of hypomagnesemia, hypercalciuria, and

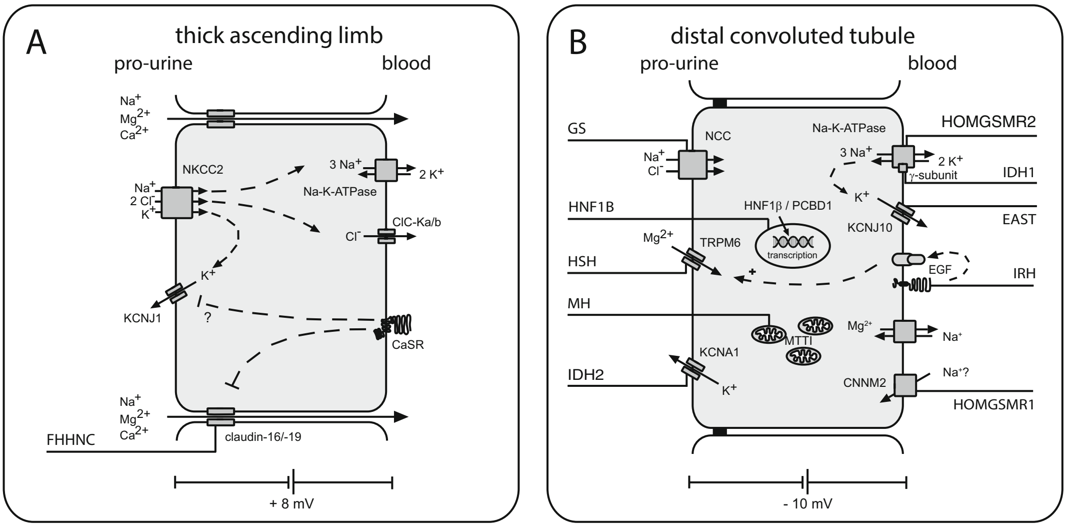

Fig. 1 Inherited disorders of magnesium reabsorption in the loop of Henle and distal convoluted tubule. (**a**) In the thick ascending limb of Henle's loop, Mg^{2+} is reabsorbed through a paracellular pathway, driven by the lumen-positive transcellular voltage generated by the transcellular reabsorption of NaCl. Mutations in the *CLDN16* and *CLDN19* genes that encode the tight junction proteins, claudin-16 and claudin-19 cause familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC). (**b**) In the distal convoluted tubule, Mg^{2+} is actively reabsorbed via the transcellular pathway involving an apical entry step through a Mg^{2+}-permeable ion channel (TRPM6) and a basolateral exit, presumably mediated by a Na^+–coupled exchange mechanism. The molecular identity of the basolateral exchange is unknown. Basolateral EGF stimulates the basolateral EGF receptor EGFR, which then increases the activity of TRPM6. Mutations of *SLC12A3* coding for NCC are responsible for Gitelman syndrome (GS). Defects in the basolateral potassium channel KCNJ10 result in EAST syndrome. Mutations in the gamma-subunit of the Na^+-K^+-ATPase (*FXYD2*) cause isolated dominant hypomagnesemia (IDH1) whereas defects in KCNA1 underlie IDH2. Isolated recessive hypomagnesemia (IRH) is caused by mutations in the *EGF* gene coding for the epidermal growth factor EGF. Mutations in the apical TRPM6 channel (*TRPM6*) cause hypomagnesemia with secondary hypocalcemia (HSH). Mutations in either the α1-subunit of the Na^+-K^+-ATPase (*ATP1A1*) or CNNM2 result in hypomagnesemia, seizures, and mental retardation (HOMGSMR1 and 2). Mitochondrial hypomagnesemia (MH) is caused by a mutation in *MTTI*

nephrocalcinosis. Additional laboratory findings include elevated PTH levels before the onset of chronic kidney disease (CKD), incomplete distal tubular acidosis, hypocitraturia, and hyperuricemia, which are present in most patients [13]. Typically, FHHNC patients clinically present during early childhood with recurrent urinary tract infections, polyuria/polydipsia, nephrolithiasis, and/or failure to thrive. Clinical signs of severe hypomagnesemia are less common. The clinical course of FHHNC is frequently complicated by the development of CKD during the first two decades of life. A considerable number of patients exhibit a marked impairment of GFR (<60 ml/min/1.73 m^2) already at the time of diagnosis and about one third of patients develops ESKD during adolescence. Due to a reduction in filtered Mg^{2+} that limits urinary Mg^{2+} losses, hypomagnesemia may completely disappear with progressive decline of GFR.

Whereas the renal phenotype is almost identical in carriers of *CLDN16* and *CLDN19* mutations, severe ocular involvement is only observed in patients with *CLDN19* mutations [6, 11, 15, 16].

Simon et al. first identified *CLDN16* (formerly known as *PCLN1*), which is mutated in many patients with FHHNC [5]. *CLDN16* codes for claudin-16, a member of the claudin family. Claudins are important components of tight junctions. The individual composition of tight junctions strands with different claudin members confers the characteristic properties of different epithelia regarding paracellular permeability

Table 1 Inherited disorders of renal magnesium handling

Disorder	OMIM #	Inheritance	Gene	Protein
Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis	248250	AR	*CLDN16*	Claudin-16, tight junction protein
Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis and ocular involvement	248190	AR	*CLDN19*	Claudin-19, tight junction protein
Gitelman syndrome	263800	AR	*SLC12A3*	NCC, Na^+-Cl^- cotransporter
EAST/SeSAME syndrome	612780	AR	*KCNJ10*	KCNJ10, potassium channel
Isolated dominant hypomagnesemia 1	154020	AD	*FXYD2*	γ-subunit of the Na^+-K^+-ATPase
Isolated dominant hypomagnesemia 2	176260	AD	*KCNA1*	KCNA1, potassium channel
Isolated recessive hypomagnesemia	611718	AR	*EGF*	Pro-EGF, epidermal growth factor
Hypomagnesemia with secondary hypocalcemia	602014	AR	*TRPM6*	TRPM6, Mg^{2+} channel
Hypomagnesemia, seizures, mental retardation 1	616418	AD/AR	*CNNM2*	CNNM2, Cyclin M2
Hypomagnesemia, seizures, mental retardation 2	618314	AD	*ATP1A1*	α1-subunit of the Na^+-K^+-ATPase
HNF1B nephropathy	137920	AD	*HNF1B*	HNF1beta, transcription factor
Transient neonatal hyperphenylalaninemia	264070	AR	*PCBD1*	PCBD1. tetrahydrobiopterin metabolism
Hypomagnesemia/metabolic syndrome	500005	Maternal	*MTTI*	Mitochondrial tRNA (Isoleucin)

and/or transepithelial resistance. In this context, a crucial role has been attributed to the first extracellular domain of the claudin protein, which is extremely variable in number and position of charged amino acid residues. Individual charges have been shown to influence paracellular ion selectivity, suggesting that claudins positioned on opposing cells forming the paracellular pathway provide charge-selective pores within the tight junction barrier.

Most mutations in *CLDN16* reported so far in FHHNC are simple missense mutations affecting the transmembrane domains and the extracellular loops with a particular clustering in the first extracellular loop. Within this domain, patients originating from Germany or Eastern European countries exhibit a common *CLDN16* mutation (L151F) due to a founder effect [13].

In FHHNC patients, progressive kidney failure is generally thought to be a consequence of massive urinary Ca^{2+} wasting and nephrocalcinosis. On the other hand, a study of a large cohort of FHHNC patients showed that the presence of *CLDN16* mutations leading to a complete loss-of-function on both alleles display a younger age at manifestation as well as a more rapid decline in kidney function compared to patients with at least one allele with residual claudin-16 function [14]. These findings support the idea that a complete lack of claudin-16 is associated with a more severe phenotype whereas a residual function delays the progression of kidney failure.

Two independent studies described a high incidence of hypercalciuria, nephrolithiasis, and/ or nephrocalcinosis in first-degree relatives of FHHNC patients [11, 13]. A subsequent study also reported a tendency toward mild hypomagnesemia in family members with heterozygous *CLDN16* mutations [18]. Thus, one might speculate that *CLDN16* mutations could be involved in the pathogenesis of idiopathic hypercalciuric stone formation.

Table 2 Clinical and biochemical characteristics of inherited disorders of renal magnesium handling

Disorder	Age at onset	Serum Mg^{2+}	Serum Ca^{2+}	Serum K^{+}	Blood pH	Urine Mg^{2+}	Urine Ca^{2+}	Nephro-calcinosis	Renal stones
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis	Childhood	↓	N	N	N or ↓	↑↑	↑↑	yes	yes
Gitelman syndrome	Adolescence	↓	N	↓	↑	↑	↓	no	no
EAST/SeSAME syndrome	Infancy	↓	N	↓	↑	↑	↓	no	no
Isolated	Childhood	↓	N	N	N	↑	↓	no	no
Isolated recessive hypomagnesemia	Childhood	↓	N	N	N	↑	N	no	no
Hypomagnesemia, seizures, mental retardation 1	Infancy to adolescence	↓↓↓	↓	N	N	↑	N	no	no
Hypomagnesemia, seizures, mental retardation 2	Infancy	↓↓↓	N	N or ↓	N	↑↑	N	no	no
HNF1B nephropathy	Broad range	↓	N	N	N	↑	↓	no	no
Hypomagnesemia after transient neonatal hyperphenylalaninemia	Adulthood	↓	N	N	↑	↑	N	no	no
Hypomagnesemia/metabolic syndrome	Adulthood	↓	N	N	N	↑	↓	no	no

A homozygous *CLDN16* mutation (T303R) affecting the C-terminal PDZ domain has been identified in two families with isolated hypercalciuria and nephrocalcinosis without disturbances in renal Mg^{2+} handling [19]. Interestingly, the hypercalciuria disappeared during follow-up and urinary Ca^{2+} levels reached normal values beyond puberty.

Molecular genetic studies in FHHNC patients with severe ocular involvement lead to the identification of mutations in a second member of the claudin family, namely, claudin-19 (encoded by *CLDN19*) [14]. The identification of *CLDN19* mutations could explain the variable ocular phenotype observed in FHHNC, because *CLDN19* defects seem to be invariably associated with severe ocular abnormalities (including severe myopia, nystagmus, or macular coloboma) [10–12]. The latter association has been named FHHNC with severe ocular involvement. In contrast, only a small subset of FHHNC patients with *CLDN16* defects displays severe myopia whereas nystagmus or colobomata have not been described [13]. The renal phenotype is very similar between these two FHHNC subtypes. Expression studies revealed that claudin-16 and claudin-19 perfectly colocalize at tight junctions of the TAL [6]. It could further be demonstrated that claudin-16 and claudin-19 functionally interact, which could increase the cation selectivity of tight junctions above that of claudin-16 alone [20]. This is most likely due to anion-blocking properties of claudin-19 preventing back diffusion of Cl^{-} anions to the tubular lumen.

Besides continuous oral Mg^{2+} supplementation, the therapy aims to reduce Ca^{2+} excretion in order to prevent the progression of nephrocalcinosis and stone formation because the degree of renal calcifications has been correlated with progression of CKD [11]. In a short-term study, thiazides have been demonstrated to effectively reduce urinary Ca^{2+} excretion in FHHNC patients [21]. However, these therapeutic strategies have not been shown yet to significantly influence the progression of CKD. Supportive therapy is important for the protection of kidney function and should include provision of sufficient fluids and effective treatment of stone formation and bacterial colonization. Kidney transplantation does not result in recurrence of the disease because the primary defect resides in the kidney.

Gitelman Syndrome

This primary salt-wasting disorder complicated by urinary Mg^{2+} wasting and hypomagnesemia is discussed in detail in ▶ Chap. 41, "Inherited Disorders of Sodium and Potassium Handling." With respect to hypomagnesemia, it is important to emphasize that hypomagnesemia is typical for Gitelman syndrome (GS) but not a universal finding, especially in young patients.

EAST/SeSAME Syndrome

In 2009, a new disease entity with autosomal recessive inheritance combining epilepsy, ataxia, sensorineural deafness, and renal NaCl wasting with/without mental retardation was described under the acronyms EAST or SeSAME syndrome [22, 23]. Patients usually present early in infancy with seizures, developmental delay, and ataxia. In addition, they exhibit a severe hearing impairment. Renal salt wasting may develop or be recognized only later during the course of the disease [24]. Closely resembling GS, the renal phenotype includes the combination of hypokalemic alkalosis, hypomagnesemia, and hypocalciuria.

EAST/SeSAME syndrome is caused by loss of function mutations in the *KCNJ10* gene encoding the inwardly rectifying K^{+}-channel KCNJ10 (Kir4.1) [22, 23]. The expression pattern of KCNJ10 fits to the disease phenotype with high expression levels in brain, the stria vascularis of the inner ear, and in the distal nephron, especially in the DCT. Here, KCNJ10 is localized at the basolateral membrane of DCT cells (Fig. 1b) and supposed to function in collaboration with Na^{+}-K^{+}-ATPase as it might allow for a recycling of K^{+} ions entering the tubular cells in a countermove for the extruded Na^{+} [23]. Loss of KCNJ10 function most likely leads to a depolarization of the basolateral membrane and thereby to a reduction of the driving force for basolateral anion channels as well as sodium-coupled exchangers. By this mechanism, KCNJ10 defects could also affect the putative Na^+/Mg^{2+} exchanger and possibly explain the Mg^{2+} wasting observed in EAST/SeSAME syndrome. Moreover, it could be demonstrated that lack of KCNJ10 decreases basolateral Cl^- conductance and results is a diminished expression of NCC in the apical membrane [25]. These results could explain the salt loss observed in EAST/SeSAME patients.

Isolated Dominant Hypomagnesemia 1

A first variant of isolated dominant hypomagnesemia (IDH1, OMIM #154020) was described in two related families by Meij et al. who discovered a missense mutation in the *FXYD2* gene, which encodes a γ-subunit of the Na^+K^+-ATPase [26]. The index patients presented with seizures during childhood (at 7 and 13 years) with serum Mg^{2+} levels of approximately 0.4 mmol/L. One patient was treated for seizures of unknown origin with antiepileptic drugs until serum Mg^{2+} levels were evaluated during adolescence. At that time mental retardation was evident [27, 28]. Serum Mg^{2+} measurements performed in additional members of both families revealed low serum Mg^{2+} levels in numerous apparently healthy individuals. Urinary Mg^{2+} measurements in affected family members revealed significant renal Mg^{2+} loss (around 5 mmol per day) despite profound hypomagnesemia. Instead, the intestinal absorption of Mg^{2+} was preserved and even stimulated in compensation for the increased renal losses. Urinary Ca^{2+} excretion rates were low in all hypomagnesemic individuals, a finding reminiscent of patients presenting with GS. In two additional families described with the same mutation, careful biochemical workup revealed a tendency toward hypokalemia and metabolic alkalosis. Muscle cramps, seizures, and chondrocalcinosis were frequent clinical findings [29].

The protein encoded by *FXYD2* is a member of a small single transmembrane protein family, which share the common amino acid motif F-X-Y-D. FXYD proteins modulate the function of the ubiquitous Na^+-K^+-ATPase, a dimeric enzyme invariably consisting of one α- and one β-subunit (Fig. 1). FXYD proteins constitute a third or γ-subunit that represents a tissue-specific regulator of the Na^+-K^+-ATPase. FXYD2 is expressed in the distal nephron, especially in the DCT and connecting tubule [30]. The FXYD2 γ-subunit increases the apparent affinity of Na^+-K^+-ATPase for ATP while decreasing its Na^+ affinity [31]. Thus, it might provide a mechanism for balancing energy utilization and maintaining appropriate salt gradients.

Expression studies of the mutant G41R-γ-subunit revealed a dominant-negative effect leading to a retention of the γ-subunit in the Golgi complex. The mechanism of a dominant negative effect is supported by the observation that individuals with a large heterozygous deletion of chromosome 11q including the *FXYD2* gene exhibit normal serum Mg^{2+} levels [32]. Urinary Mg^{2+} wasting together with the expression pattern of the *FXYD2* gene indicate defective transcellular Mg^{2+} reabsorption in the DCT. The exact mechanism causing increased urinary Mg^{2+} excretion is yet to be determined.

Isolated Dominant Hypomagnesemia 2

Genetic heterogeneity in IDH was demonstrated by the identification of a heterozygous missense mutation in the *KCNA1* gene, which encodes the voltage-gated potassium channel KCNA1 (Kv1.1) [33]. The observed clinical picture includes early onset muscle cramps, tetany, tremor, and muscle weakness. Biochemical workup revealed a renal Mg^{2+} leak without alterations in renal Ca^{2+} handling. Mutations in *KCNA1* had previously been identified in patients with episodic ataxia with myokymia (OMIM 160120), a neurologic disorder characterized by an intermittent appearance of incoordination and imbalance as well as myokymia, an involuntary, spontaneous, and localized trembling of muscles. In addition to muscle cramps and tetany attributed to Mg^{2+} deficiency, these symptoms were also present in members of the aforementioned family with hypomagnesemia.

The mutation in *KCNA1* described in hypomagnesemic patients leads to a nonconservative amino acid exchange (N255D) in KCNA1. Functional voltage-gated K^+-channels of the KCNA family are composed of tetramers. Coexpression of the N255D mutant with wild-type KCNA1 subunits revealed a dominant negative effect that rather seems to result from impaired channel gating since trafficking to the plasma membrane is preserved [34].

KCNA1 expression was demonstrated in DCT cells at the apical membrane (Fig. 1b). Because of its colocalization with TRPM6, a model was proposed in which KCNA1 allows for hyperpolarization of the apical membrane of DCT cells as a prerequisite for TRPM6-mediated Mg^{2+} entry (Fig. 1b).

Isolated Recessive Hypomagnesemia

Geven and colleagues reported a form of isolated recessive hypomagnesemia (IRH) in a consanguineous family [35]. Two affected girls presented with generalized seizures during infancy. Possibly related to late diagnosis, both patients also exhibited neurodevelopmental deficits. Clinical and laboratory workup at 4 and 8 years of age, respectively, revealed serum Mg^{2+} levels around 0.5–0.6 mmol/L with no other associated electrolyte abnormalities. Both patients exhibited renal Mg^{2+} excretion of 3–6 mmol per day despite hypomagnesemia confirming renal Mg^{2+} wasting. Renal Ca^{2+} excretion rates in IRH are within the normal range.

As the underlying molecular defect, a homozygous P1070L mutation in the *EGF* gene encoding the epidermal growth factor EGF was identified [36]. The EGF protein is expressed in the DCT, and its binding to the receptor EGFR is essential for the function of the TRPM6 channel (Fig. 1b). The mutation is located in the cytosolic C-terminal terminus within a sorting motif (PXXP), which is necessary for the trafficking of EGF to the basolateral membrane. Expression studies demonstrated that mutant pro-EGF retains EGF secretion to the apical membrane but does not reach the EGF receptor in the basolateral membrane, resulting in dysfunction of TRPM6 [36]. Even if IRH seems to be an extremely rare disease phenotype, the identification of EGF mutations is important because this is the first autocrine/paracrine magnesiotropic hormone known at the molecular level.

Hypomagnesemia with Secondary Hypocalcemia

Hypomagnesemia with secondary hypocalcemia (HSH) is a rare recessive disorder that presents in early infancy with generalized seizures or other clinical signs of increased neuromuscular excitability [37]. Biochemical abnormalities include extremely low serum Mg^{2+} (about 0.2 mmol/L) and low serum Ca^{2+} levels. Hypocalcemia is thought to result from an impaired synthesis and/or release of PTH in the presence of severe hypomagnesemia [38]. The mechanism leading to hypocalcemia is not completely clear, but severe hypomagnesemia results in impaired synthesis and/or release of PTH [39]. The hypocalcemia observed in HSH does not respond to therapy with Ca^{2+} or vitamin D. Relief of clinical symptoms, normocalcemia, and normalization of PTH levels are only achieved by administration of high doses of Mg^{2+} [40]. Serum magnesium levels usually remain in the subnormal range despite adequate treatment but enable an undisturbed physical and mental development in most patients. However, delayed diagnosis or noncompliance with treatment can be fatal or result in permanent neurological damage.

Transport studies in HSH patients pointed to a primary defect in intestinal Mg^{2+} absorption [41, 42]. However, in some patients an additional renal leak for Mg^{2+} was suspected [43].

In 2002, recessive loss of function mutations in TRPM6 encoding a member of the transient receptor potential (TRP) family of ion channels was discovered as the underlying genetic defect [44, 45]. To date, mutations in *TRPM6* have been identified in more than 50 families affected by HSH [44–48]. The mutational spectrum mainly comprises truncating mutations. In addition, a number of missense mutations have been described for which functional analyses also indicated a complete loss-of-function [44, 46, 48, 49].

TRPM6 encodes a member of the transient receptor potential (TRP) family of cation ion channels. It is expressed along the intestine as well as in the DCT of the kidney [44]. In the intestine, intraluminal Mg^{2+} concentrations and rates of Mg^{2+} absorption show a curvilinear relationship presumably reflecting two transport processes working in parallel: an active and saturable transcellular transport essential at low intraluminal Mg^{2+} concentrations and a passive paracellular Mg^{2+} absorption gaining importance at higher intraluminal Mg^{2+} concentrations [1]. The observation that in HSH patients the substitution of high oral doses of Mg^{2+} achieves at least subnormal serum Mg^{2+} levels supports the idea of two independent intestinal transport systems for Mg^{2+}. TRPM6 probably represents a molecular component of active transcellular Mg^{2+} transport. An increased intraluminal Mg^{2+} concentration (by increased oral intake) enables to compensate for the defect in active transcellular transport by increasing absorption via the passive paracellular pathway.

The detection of TRPM6 expression in the DCT confirmed the hypothesis of an additional role of renal Mg^{2+} wasting for the pathogenesis of HSH [50]. This was also supported by intravenous Mg^{2+} loading tests in HSH patients, which disclosed a considerable renal Mg^{2+} leak albeit still being hypomagnesemic [45].

TRPM6 is thought to participate in the formation of magnesium-permeable ion channels in the apical membrane of DCT cells (Fig. 1b). As electrophysiological experiments yielded controversial data, it is still unclear if TRPM6 constitutes homomeric ion channels in vivo or is involved in the formation of heterotetramers with its close homologue TRPM7 [51–53].

Hypomagnesemia, Refractory Seizures, and Mental Retardation 1

Another form of hereditary Mg^{2+} wasting could be linked to mutations in *CNNM2* encoding the transmembrane protein CNNM2 or Cyclin M2 [54].

Stuiver et al. identified heterozygous CNNM2 mutations in two families with dominant hypomagnesemia [54]. Clinical symptoms and age at manifestation were variable with symptoms ranging from seizures in early childhood to muscle weakness, vertigo, and headache during adolescence. Other heterozygous carriers from both families even remained asymptomatic. Except for hypomagnesemia (Mg^{2+} of 0.36 to 0.51 mmol/L in symptomatic patients), no additional biochemical abnormalities were reported. Whereas a truncating frame-shift mutation was identified in one of the described families, affected individuals of the second family were found to carry a missense mutation leading to a nonconservative amino acid exchange in CNNM2 [54]. Patch clamp analyses revealed a significant reduction in Mg^{2+}-sensitive, inwardly rectifying Na^{+} currents for this missense mutant [54].

The phenotypic and genetic spectrum of CNNM2-associated disease was broadened by the identification of a recessive mutation in a family with parental consanguinity [55]. The two affected siblings exhibited hypomagnesemia (~0.5 mmol/L) and a severe degree of psychomotor retardation. Magnetic resonance imaging of the central nervous system in one of the patients revealed widened outer cerebrospinal liquor spaces as well as myelinization defects. The finding of a severe neurological phenotype caused by bi-allelic *CNNM2* mutations was substantiated by a second study describing a second homozygous mutation in *CNNM2* in a patient with a similar clinical picture comprising refractory epilepsy, microcephaly, global developmental delay, and intellectual disability [56]. Later, the description of a larger cohort of patients enabled a more detailed clinical description of the phenotype associated with heterozygous CNNM2 defects [57]. Serum Mg^{2+} levels are typically in the range of 0.5–0.6 mmol/L, insufficiently respond to oral Mg^{2+} supplementation, and remain in the subnormal range. Patients typically manifest with cerebral seizures in infancy, but seizures subside during follow-up in most patients. However, patients uniformly show a mild to moderate degree of neurodevelopmental delay with a disturbed speech development and dysarthria as prominent features. Finally, a considerable number of patients of both genders develops obesity (BMI >97th percentile) as the most prominent extraneurological symptom [57].

The precise physiological function of CNNM2 still remains enigmatic. CNNM2 is ubiquitously expressed in mammalian tissues, most prominently in kidney, brain, and lung [58, 59]. In the kidney, CNNM2 expression was demonstrated at the basolateral membrane of TAL and DCT.

Although CNNM2 had been proposed as a Mg^{2+}-transporter after overexpression studies in *Xenopus* oocytes [60], Mg^{2+}-transport could not be directly measured in mammalian cells using patch clamp analyses [54]. In-silico modeling of CNNM2 protein domains identified a Mg^{2+}-ATP-binding site suggesting a putative role in Mg^{2+} sensing [59]. Using stable Mg^{2+}-isotopes, an increase in cellular Mg^{2+}-uptake could be demonstrated after overexpression of CNNM2, an effect that was abrogated by the introduction of pathogenic mutations identified in hypomagnesemic patients [55, 57]. Finally, CNNM2 function was studied in the zebrafish model. Here, knockdown of CNNM2 expression leads to a decrease in whole-body Mg^{2+} as well as developmental defects of the central nervous system in line with human disease [55]. Further studies will be needed to clarify if CNNM2 serves as a Mg^{2+}-sensor or if it by itself is able to transport Mg^{2+}.

Hypomagnesemia, Refractory Seizures, and Mental Retardation 2

Heterozygous de novo mutations in *ATP1A1* encoding the α1-subunit of Na^+K^+-ATPase have recently been described in three children with severe hypomagnesemia due to renal Mg^{2+} wasting [61]. Affected individuals presented during infancy with seizures that were not responsive to antiepileptic medication and did not cessate upon Mg^{2+} supplementation. All three children developed a significant degree of mental retardation and global developmental delay. Of note, serum Mg^{2+} concentrations remained low despite adequate supplementation.

The α1-subunit (ATP1A1) is one of four different α-subunits of Na^+K^+-ATPase in human, but it represents the exclusive α-subunit in kidney. Along the kidney tubule, the DCT represents the segment with the highest energy consumption and density of Na^+K^+-ATPase expression [62]. Here, basolaterally expressed Na^+K^+-ATPase generates favorable electrochemical gradients for transcellular NaCl and Mg^{2+} reabsorption. The critical role of Na^+K^+-ATPase for Mg^{2+} reabsorption had previously been demonstrated by discovery of mutations in its γ-subunit (*FXYD2*) in patients with isolated dominant hypomagnesemia (see above). In the central nervous system, the α1-subunit is ubiquitously expressed and thought to maintain neuronal housekeeping functions [63].

The ATP1A1 mutations discovered in hypomagnesemic children were shown to not only lead to a loss of ATPase function, but also to result in abnormal ion permeabilities and leak currents [61]. Therefore, the severe hypomagnesemia phenotype might not be the result of a simple ATP1A1 haploinsufficiency. Whereas a homozygous loss of ATP1A1 function is not compatible with life, heterozygous germline ATP1A1 mutations have also been described in patients with Charcot-Marie-Tooth disease type 2 and hereditary spastic paraplegia [64, 65].

HNF1B Nephropathy

Hepatocyte nuclear factor 1β (HNF1B) is a transcription factor, which plays a critical role in the development of the kidney and the pancreas. Heterozygous mutations in *HNF1B* were initially reported in maturity-onset diabetes of the young (MODY5) [66]. *HNF1B* mutations occur in the heterozygous state, either inherited or de novo, and comprise point mutations as well as whole gene deletions [67]. Subsequent reports described an association with developmental kidney disease. The kidney anomalies are highly variable comprising enlarged hyperechogenic kidneys, multicystic kidney disease, renal agenesis, renal hypoplasia, cystic dysplasia, as well as hyperuricemic nephropathy. The association of MODY and cystic kidneys led to the term renal cysts and diabetes syndrome (RCAD) [68]. However, this denomination might be misleading because neither the renal cystic phenotype nor the diabetes is a constant clinical finding [69]. For this reason, the new term HNF1B nephropathy has been introduced.

Approximately 50% of patients develop hypomagnesemia due to impaired renal Mg^{2+} reabsorption [70]. Renal Mg^{2+} wasting is accompanied by hypocalciuria indicating an involvement of the DCT [70]. A follow-up study investigated the tubulopathy associated with HNF1B nephropathy in more detail and also demonstrated hypokalemia and metabolic alkalosis in addition to hypomagnesemia and hypocalciuria pointing to a general disturbance of the DCT [71]. As another study identified hypomagnesemia only in a smaller percentage of affected children (25%), it was shown that this phenotypic feature of HNF1B nephropathy represents an age-dependent phenomenon and develops over time [72]. This assumption was supported by a Polish study that reported an increasing fraction of hypomagnesemic HNF1B patients during follow-up [73].

Transient Neonatal Hyperphenylalaninemia

Renal Mg^{2+} wasting has recently been described in transient neonatal hyperphenylalaninemia due to recessive mutations in the *PCBD1* gene [74]. Affected individuals were shown to develop hypomagnesemia and a MODY type diabetes at adult age. Functional studies revealed that PCBD1 is an essential dimerization cofactor of HNF1B. It was demonstrated that a defective dimerization of PCBD1 with HNF1B abrogated the HNF1B-mediated stimulation of *FXYD2* promoter activity in the DCT [74].

Mitochondrial Hypomagnesemia

A mutation in the mitochondrially encoded isoleucine tRNA gene, $tRNA^{Ile}$ or MTTI, related to hypomagnesemia has been discovered in a large Caucasian kindred [75]. An extensive clinical evaluation of this family was prompted after the discovery of hypomagnesemia in the index patient, leading to the characterization of mitochondrial hypomagnesemia. Indeed, pedigree analysis was compatible with mitochondrial inheritance as the phenotype was exclusively transmitted by affected females. The phenotype includes hypomagnesemia, hypercholesterolemia, and hypertension. Of the adults on the maternal lineage, the majority of offspring exhibited at least one of the mentioned symptoms, approximately half of the individuals showed a combination of two or more symptoms, and around 1/6 had all three features. Serum Mg^{2+} levels of family members on the maternal lineage varied greatly from ~0.3 to ~1.0 mmol/L with approximately 50% of individuals being hypomagnesemic. The hypomagnesemic individuals showed higher fractional excretion rates (median around 7.5%) than their normomagnesemic relatives (median around 3%) clearly pointing to renal Mg^{2+} wasting as causative for hypomagnesemia. Interestingly, hypomagnesemia was accompanied by decreased urinary Ca^{2+} levels, a finding pointing to the DCT as the affected tubular segment.

As ATP consumption along the tubule is highest in the DCT, the authors speculate about an impaired energy metabolism of DCT cells as a consequence of the mitochondrial defect, which in turn could lead to disturbed transcellular Mg^{2+} reabsorption [75]. Further studies in these patients might help to better understand the mechanism of distal tubular Mg^{2+} wasting in this disease.

Management of Hypomagnesemia/Magnesium Deficiency

The main goal of Mg^{2+} substitution in hypomagnesemic patients is the relief of clinical symptoms. In most cases, especially in primary Mg^{2+} wasting diseases, normal levels cannot be achieved by oral substitution without considerable gastrointestinal side effects. The route of administration depends on the severity of clinical symptoms. Acute intravenous infusions should be reserved for patients with severe symptoms, i.e., with cerebral seizures [76]. Especially in children, painful intramuscular injections should be avoided. In infants and children, the starting dose is 20–50 mg Mg^{2+} sulfate (0.1–0.2 mmol Mg^{2+}) per kilogram body weight. Mg^{2+} sulfate should be given slowly intravenously (over 20 min). The maximum dose for adults is 2 g of Mg^{2+} sulfate. Single doses can be repeated every 6–8 h or followed by continous infusion of 100–200 mg Mg^{2+} sulfate (0.4–0.8 mmol Mg^{2+}) per kilogram body weight per day [77]. During Mg^{2+} infusion, close monitoring of cardiorespiratory function is important and Ca^{2+} gluconate should be available as an antidote. The assessment of kidney function is also mandatory.

Asymptomatic hypomagnesemia or chronic Mg^{2+} deficiency should be treated with oral Mg^{2+} substitution. In children, 10–20 mg Mg^{2+} (0.4–0.8 mmol) per kg body weight given three to four times a day has been recommended to correct hypomagnesemia [78]. Of note, the solubility, intestinal absorption, and side effects considerably differ depending on the Mg^{2+} salt used for oral therapy. The bioavailability and pharmacokinetics of different Mg^{2+} salts have been reviewed in [79]. With respect to solubility, intestinal absorption, and bioavailability, organic Mg^{2+} salts (e.g., citrate or aspartate) appear most suitable for oral substitution. Moreover, the laxative effect of organic Mg^{2+} salts seems to be less pronounced compared to inorganic salts.

The use of certain diuretics has been proposed for the reduction of renal Mg^{2+} excretion. Both, K^+-sparing diuretics and aldosterone antagonists, exert Mg^{2+}-sparing effects [80, 81]. Their beneficial effect on renal Mg^{2+} excretion, serum Mg^{2+} levels and clinical symptoms is well documented in hereditary Mg^{2+}-wasting diseases [82].

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Inherited Disorders of Renal Calcium Handling

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Karl Peter Schlingmann and Martin Konrad

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Abstract

Human calcium homeostasis is maintained by a complex interplay of different hormonal systems controlling intestinal calcium uptake, renal calcium excretion, and calcium transport in bone and soft tissues. Changes in renal calcium handling may either reflect a renal compensation for disturbances in body calcium homeostasis or its hormonal regulation, or a primary defect in renal tubular calcium transport. Irrespective of the underlying etiology, hypercalciuria represents a major risk factor for renal calcifications and kidney stone disease requiring a thorough diagnostic workup. Especially in infants and young children, hereditary disorders of calcium metabolism and tubular calcium reabsorption have to be considered in the differential diagnosis. The elucidation of the underlying genetic etiology might directly affect the management of affected children.

Keywords

Calcium · Hypocalcemia · Hypercalcemia · Hypercalciuria · Nephrocalcinosis · Vitamin D · Hypervitaminosis D

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Calcium Physiology

Elemental calcium constitutes around 1 kg of adult human body mass of which 99% are bound in bone. Extracellular fluid only contains approximately 1 g of calcium. In serum, around 50 % of calcium are present in the free ionized form (Ca^{2+}), ~35 % are protein-bound, and ~15 % are complexed to bicarbonate, citrate, or phosphate. The physiological range for blood calcium equals 1.1 to 1.35 mmol/L for free, ionized Ca^{2+} and 2.2 to 2.6 mmol/L for total calcium (in the presence of physiological whole protein levels) [1]. Only the free, ionized fraction is responsible for the biological calcium effects.

According to recommendations of national boards of nutrition, 1000 to 1200 mg of calcium should be taken up daily with a normal diet [2, 3]. The intestine reabsorbs approximately 25 to 33 % of the nutritional calcium content [4]. In the kidney, approximately 800 mg of ionized Ca^{2+} is filtered daily in the glomeruli of which 99 % is reabsorbed along the tubule. Only ~10 mg (~0.1mg/kg/day) is finally excreted in the urine.

Blood calcium levels are kept within a narrow physiological range by the concerted action of different hormonal systems that control intestinal calcium uptake, renal calcium excretion, and calcium transport in bone and soft tissues. These comprise the parathyroid gland with parathyroid hormone (PTH), active 1,25-$(OH)_2$-vitamin D_3, and the bone-derived, phosphaturic hormone FGF-23 [5].

All three systems are tightly linked and influence each other's activity. PTH increases intestinal calcium absorption and calcium release from bone, and it promotes renal calcium reabsorption while stimulating renal phosphate excretion. In contrast, active 1,25-$(OH)_2$-vitamin D_3 promotes intestinal calcium and phosphate absorption as well as renal calcium and phosphate conservation thereby ensuring sufficient calcium and phosphate supply for bone mineralization. Finally, FGF-23 together with its co-factor klotho increases renal phosphate excretion by inhibiting proximal tubular phosphate reabsorption [5, 6]. In addition, FGF-23 also negatively regulates vitamin D by inhibiting its activation and promoting its degradation. The common aim of this hormonal interplay is to keep extracellular calcium levels constant while supplying sufficient amounts of calcium for soft tissues and bone mineralization.

At the glomerulus, the ionized fraction of serum calcium is freely filtered. The majority of filtered calcium (60–70%) is already reabsorbed in the proximal tubule (Fig. 1) [7]. Classically, calcium transport in the proximal tubule is considered to occur via the paracellular pathway driven by active transcellular sodium reabsorption and paracellular water flow [8]. Apical uptake of sodium into the proximal tubular cell is achieved via the sodium proton exchanger NHE3 (*SLC9A3*) and followed by a basolateral extrusion via Na-K-ATPase [9]. The paracellular space in the proximal tubule has a high permeability for ions and water that is conferred by tight junctions composed of claudin-2, claudin-10, and claudin-12 [10, 11]. Interestingly, recent data also indicate transcellular calcium transport in the proximal tubule involving apically expressed Calcium-sensing receptor (CaSR) and the calcium-permeable ion channel TRPC3 (transient receptor potential family C member 3) [12].

Around 25% of filtered calcium is reabsorbed in the thick ascending limb (TAL) of the loop of Henle. Here, calcium transport is passive and paracellular in nature and occurs together with magnesium (see below). It is driven by a lumen-positive transepithelial voltage generated by active transcellular salt reabsorption. Next to changes in transcellular sodium chloride reabsorption, the permeability and cation selectivity of the paracellular pathway play a critical role in determining calcium and magnesium reabsorption. Paracellular tight junctions in the TAL are composed of a complex set of claudin proteins conferring different properties: claudin-16 and claudin-19 facilitate paracellular calcium and magnesium transport, but also influence sodium permeability while sealing the paracellular space for chloride [13]. Furthermore, paracellular movement of calcium and magnesium is modified by claudin-10 which is thought to form a paracellular sodium pore [14]. Paracellular calcium and magnesium transport in the

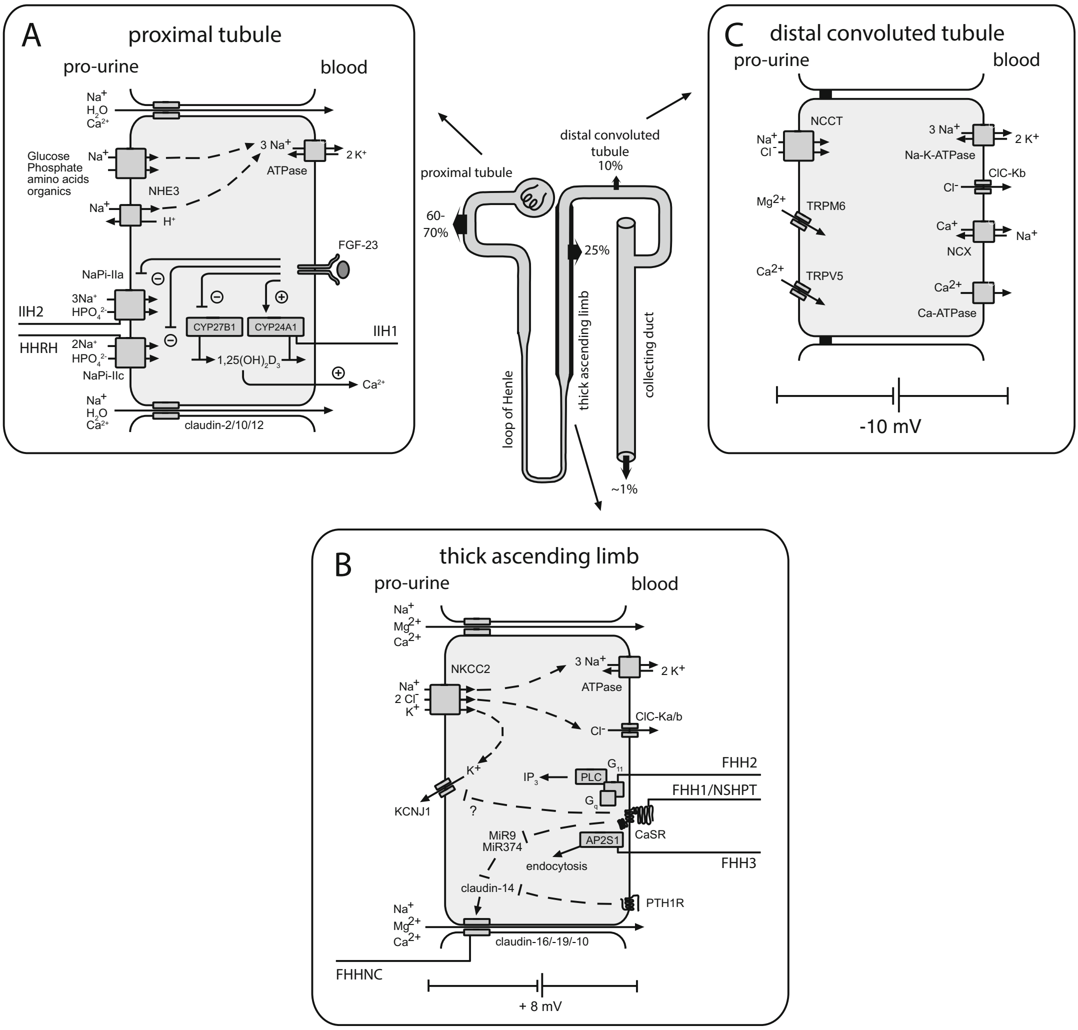
A
proximal tubule
pro-urine
blood
Na+ H2O Ca2+
Glucose Phosphate amino acids organics
Na+
3 Na+
2 K+
ATPase
NHE3
Na+
H+
FGF-23
NaPi-IIa
3Na+ HPO4 2-
IIH2
HHRH
2Na+ HPO4 2-
NaPi-IIc
CYP27B1
CYP24A1
IIH1
1,25(OH)2D3
Ca2+
Na+ H2O Ca2+
claudin-2/10/12
proximal tubule
60-70%
distal convoluted tubule 10%
25%
loop of Henle
thick ascending limb
collecting duct
~1%
C
distal convoluted tubule
pro-urine
blood
NCCT
Na+ Cl-
3 Na+
2 K+
Na-K-ATPase
ClC-Kb
Cl-
Mg2+
TRPM6
Ca+
Na+
NCX
Ca2+
TRPV5
Ca2+
Ca-ATPase
-10 mV
B
thick ascending limb
pro-urine
blood
Na+ Mg2+ Ca2+
NKCC2
Na+ 2 Cl- K+
3 Na+
2 K+
ATPase
ClC-Ka/b
Cl-
K+
IP3
PLC
G11
FHH2
FHH1/NSHPT
Gq
KCNJ1
?
CaSR
MiR9 MiR374
AP2S1
FHH3
endocytosis
claudin-14
PTH1R
Na+ Mg2+ Ca2+
claudin-16/-19/-10
FHHNC
+ 8 mV

Fig. 1 Calcium reabsorption along the kidney tubule and inherited disorders affecting calcium metabolism and renal calcium handling (A) In the proximal tubule, calcium is reabsorbed via a paracellular pathway through tight junctions containing claudin-2. Moreover, the enzymes responsible for vitamin D activation, 1α-hydroxylase (CYP27B1), and vitamin D inactivation, 24-hydroxylase (CYP24A1), are expressed in proximal tubular cells and are functionally linked to phosphate reabsorption via NaPi-IIa (SLC34A1) and NaPi-IIc (SLC34A3). (B) In the thick ascending limb (TAL) of Henle's loop, calcium is reabsorbed together with magnesium through paracellular tight junctions composed by claudin-16 and -19. This process is negatively regulated by the basolateral Calcium-sensing receptor (CaSR) and PTH. Mutations in the CaSR and its associated proteins cause changes in calcium metabolism (see text for details). (C) In the distal convoluted tubule (DCT), calcium is actively reabsorbed via the transcellular pathway involving an apical entry through TRPV5 and a basolateral exit via Ca^{2+}-ATPase and Na^{+}-Ca^{2+}-exchanger (NCX). There are no known monogenic disorders affecting calcium reabsorption in the DCT

TAL is regulated by a complex network involving claudin-14 as well as the CaSR and PTH receptor, both expressed at the basolateral membrane [15–17]. Claudin-14 interacts with claudin-16 and diminishes paracellular cation permeability *in vitro*. Under physiologic conditions, the expression of claudin-14 is suppressed via a microRNA pathway [18]. By activation of the CaSR, extracellular calcium is able to relieve this suppression, induce claudin-14 expression, and inhibit paracellular divalent cation reabsorption [16]. The CaSR is a guanine nucleotide-binding protein (G-protein)-coupled receptor that stimulates phospholipase C through G-proteins G_q and G_{11} resulting in the production of inositol 1,4,5-trisphosphate. This in turn leads to a reduction in PTH secretion from the parathyroid gland as well as increased urinary calcium and magnesium excretions [19]. Finally, claudin-14 expression and therefore paracellular cation transport are directly regulated by PTH via basolaterally expressed PTH-receptor PTH1R [17].

The distal convoluted tubule (DCT) only reabsorbs a small proportion of filtered calcium (10 - 15 %); however, as for magnesium, the DCT determines the final urinary excretion as there is no calcium reabsorption beyond this segment. Transcellular calcium reabsorption in the DCT is a sequential process of three steps: apical entry into the epithelial cell through calcium-permeable ion channels (TRPV5), binding to calbindin-D_{28k} for diffusion through the cytoplasm, and basolateral exit through either a Ca^{2+}-ATPase (PMCA1b, *ATP2B1*), or a Na^{+}-Ca^{2+}-exchanger (NCX1, *SLC8A1*) [20]. Transcellular calcium reabsorption in the DCT is critically influenced by PTH and active 1,25-OH_2-vit D_3 that regulate the expression levels of all of these transcellular transport proteins [21]. In addition, calcium (as well as magnesium) handling in the DCT appears to depend on dietary potassium and the rate of active transcellular salt reabsorption [22, 23].

Disturbances of Calcium Homeostasis

Disturbances in calcium metabolism comprise states of calcium deficiency as well as calcium excess usually detected in the form of hypo- or hypercalcemia. Whereas hypocalcemia represents a more common finding and is usually apparent by typical clinical signs and symptoms, hypercalcemia in infancy and childhood is a rare event and may remain unrecognized as clinical symptoms are rather unspecific. Here, we focus on hereditary disorders of calcium metabolism. These typically manifest with characteristic changes in serum and urine calcium levels. As for acquired disorders of calcium homeostasis, the diagnostic work-up, next to the parallel measurement of serum and urine electrolytes and creatinine, comprises the determination of parathyroid hormone (PTH) and vitamin D metabolites.

Hypercalciuria

An increased urinary calcium excretion may disclose [1] an appropriate renal response to excessive amounts of calcium from circulation and [2] an inappropriate renal Ca^{2+} conservation with or without apparent changes in body calcium homeostasis and in its hormonal regulation. The assessment of urinary calcium excretion is commonly performed by either 24h urine collections and calculation of daily calcium excretion per kg body weight or by calculation of the calcium/creatinine ratio from spot urine samples. For reference values for renal calcium excretion in infants and children, please refer to Matos et al. [24].

Hypercalciuria is the most common risk factor for nephrocalcinosis and kidney stone formation in children [25]. The etiology is assumed to be multifactorial with a complex interaction of environmental and genetic factors. Still, despite an increased knowledge in the underlying genetic causes, the majority of patients are classified as having idiopathic hypercalciuria. A detailed overview on hypercalciuria and its role in the etiology of nephrocalcinosis and kidney stone disease is also provided in chapter “Urolithiasis and Nephrocalcinosis”.

In the past two decades, genetic studies have been able to identify variants in an increasing number of genes and proteins involved in tubular calcium reabsorption in patients with hypercalciuria, nephrocalcinosis and kidney stone disease. Classic examples of a primary disturbance of renal tubular calcium (and magnesium) reabsorption are hereditary defects in the two tight junction proteins claudin-16 and -19 in patients with familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) (HOMG3, OMIM #248250; HOMG5, OMIM #248190) as well as activating and deactivating mutations in the CaSR producing mirror images of disturbed calcium homeostasis

(ADH1, OMIM #601198; FHH1, OMIM #144980; NSHPT, OMIM #239200). Whereas FHHNC is discussed in detail in chapter on “inherited disorders of renal magnesium handling”, the hereditary defects of CaSR function are presented below in the respective paragraphs on hypo- and hypercalcemia.

Established disorders associated with hypercalciuria and nephrocalcinosis affecting proximal tubular calcium reabsorption comprise different forms of hereditary renal Fanconi syndrome (FRTS1 (GATM), OMIM 134600; FRTS3 (EHHADH), OMIM 615605; FRTS4 (HNF4A), OMIM 616026), Dent’s disease (DENT1, OMIM #300009; DENT2, OMIM #300555), Lowe syndrome (OMIM #309000), nephropathic cystinosis (CTNS, OMIM 219800), Fanconi Bickel syndrome (FBS, OMIM 227810), Tyrosinemia (TYRSN1, OMIM 276700), Galactosemia (GALAC1, OMIM 230400), as well as primary mitochondrial disorders [26]. These disorders are discussed in more detail elsewhere.

As paracellular calcium and magnesium reabsorption in the TAL depends on transepithelial voltage generated by active transcellular salt reabsorption, different types of Bartter syndrome affecting salt reabsorption in the TAL typically lead to hypercalciuria and nephrocalcinosis (Table 1) (see chapter “Inherited disorders of sodium and potassium handling”).

Finally, hypercalciuria and nephrocalcinosis are commonly observed in patients with different forms of distal renal tubular acidosis (RTA). Hypercalciuria in dRTA is typically believed to result from an increased release of calcium from bone; however, metabolic acidosis also directly affects renal tubular calcium handling. The effects of changes in systemic acid-base status on paracellular transport processes in the proximal tubule and TAL have not been fully elucidated. An increased serum load of bone-derived calcium could theoretically inhibits tubular calcium reabsorption via the CaSR and increases claudin-14 expression in paracellular tight junctions in the TAL [27]. Moreover, metabolic acidosis has been shown to directly downregulate the expression of calcium (and magnesium) transport proteins in the DCT [28]. The different forms of renal tubular acidosis are presented in ▶ Chap. 38, “Renal Tubular Acidosis”.

An overview of hereditary disorders leading to an increased urinary excretion of calcium, nephrocalcinosis, and kidney stone disease is provided in Table 1.

A recent study directly implicates defective paracellular calcium reabsorption in the proximal tubule in the pathogenesis of hypercalciuria and renal calcifications as deletion of claudin-2 in mice was shown to result in hypercalciuria and renal calcifications [29]. The same study also identified a potentially pathogenic heterozygous variant in *CLDN2* in a family with multiple members affected by kidney stones and associated common genetic variants near the *CLDN2* locus with kidney stone risk [29].

Surprisingly and in contrast to calcium and magnesium reabsorption in the TAL as well as magnesium reabsorption in the DCT (see below), a primary defect in transcellular calcium reabsorption in the DCT could not yet be linked to monogenic human disease. Deletion of the epithelial calcium channel TRPV5 in mice was shown to result in severe hypercalciuria, compensatory intestinal hyperabsorption of calcium, and reduced bone thickness [30]. Later, a TRPV5 missense mutation was identified in a mouse model with dominant hypercalciuria [31]. In contrast, homozygous knock-out of basolateral NCX1 (SLC8A1) and PMCA1b (ATP2B1) in mice was shown to be embryonically lethal [32, 33].

Hypocalcemia

The etiology of calcium deficiency and hypocalcemia can most easily be divided into different entities according to the serum level of PTH [34]. Low levels of PTH indicate a primary dysfunction of the parathyroid gland with impaired or absent PTH production or release. Hereditary disorders involving an abnormal parathyroid gland function include primary hypoparathyroidism due to mutations in the *PTH* gene (isolated familial hypoparathyroidism 1, OMIM #146200), due to DiGeorge syndrome, caused by microdeletions on chromosome 22q11 (OMIM #188400), or due to

Table 1 Inherited disorders associated with hypercalciuria and nephrocalcinosis

Disorder	OMIM #	Inheritance	Gene	Protein
Renal Fanconi Syndrome 1 (FRTS1)	134600	AD	*GATM*	Arg-Gly-Amidinotransferase
Renal Fanconi Syndrome 3 (FRTS3)	615605	AD	*EHHADH*	Enoyl-CoA-Hydratase
Renal Fanconi Syndrome 4 (FRTS4)	616026	AD	*HNF4A*	Hepatocyte-Nuclear Factor 4α
Dent's disease 1	300009	XLR	*CLCN5*	Chloride Channel ClC-5
Dent's disease 2/Lowe syndrome	300555 309000	XLR	*OCRL*	Inositol-Polyphosphate-5-Phosphatase
Nephropathic cystinosis	219800	AR	*CTNS*	Cystinosin
Fanconi-Bickel syndrome	227810	AR	*SLC2A2*	Facilitated glucose transporter 2
Tyrosinemia	276700	AR	*FAH*	Fumarylacetoacetate hydrolase
Galactosemia	230400	AR	*GALT*	Galactose-1P-Uridyltransferase
Wilson disease	277900	AR	*ATP7B*	Cu-transporting ATPase
Infantile hypercalcemia 1 (IIH1), nephrocalcinosis, calcium nephrolithiasis	143880	AR, AD	*CYP24A1*	25-OH-vitamin D_3-24-hydroxylase
Infantile hypercalcemia 2 (IIH2), nephrocalcinosis, calcium nephrolithiasis	616963	AR, AD	*SLC34A1*	Na-Po_4 Co-transporter NaPi-IIa
Hypophosphatemic rickets with hypercalciuria (HHRH), nephrocalcinosis, calcium nephrolithiasis	241530	AR, AD	*SLC34A3*	Na-Po_4 Co-transporter NaPi-IIc
Williams-Beuren syndrome	194050	AD	*7q11.23*	Contiguous gene deletion syndrome
Bartter syndrome type 1	601678	AR	*SLC12A1*	Na-K-2Cl cotransporter
Bartter syndrome type 2	241200	AR	*KCNJ1*	Kir1.1, ROMK potassium channel
Bartter syndrome type 3	607364	AR	*CLCNKB*	Chloride channel ClC-Kb
Bartter syndrome type 4A	602522	AR	*BSND*	Barttin ClC-K subunit
Bartter syndrome type 4B	613090	AR	*CLCNKA/B*	Chloride channel ClC-Ka and ClC-Kb
Bartter syndrome type 5, transient	300971	XLR	*MAGED2*	Melanoma antigen D2
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis (FHHNC)	248250	AR	*CLDN16*	Claudin-16 (paracellin-1), tight junction protein
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis (FHHNC) and severe ocular involvement	248190	AR	*CLDN19*	Claudin-19, tight junction protein
Autosomal dominant hypoparathyroidism 1 (ADH1)	601198	AD	*CASR*	Calcium-sensing receptor

(continued)

Table 1 (continued)

Disorder	OMIM #	Inheritance	Gene	Protein
Distal renal tubular acidosis 1	179800	AD	*SLC4A1*	Anion exchanger 1 (AE1)
Distal renal tubular acidosis 2 with deafness	267300	AR	*ATP6V1B1*	H^+-ATPase subunit V1
Distal renal tubular acidosis 3 with/without deafness	602722	AR	*ATP6V0A4*	H^+-ATPase subunit A4
Distal renal tubular acidosis with enlarged aqueduct	600791	AR	*FOXI1*	Forkhead transcription regulator I1
Proximal renal tubular acidosis	604278	AR	*SLC4A4*	Na-HCO_3 cotransporter 1 (NBC1)

HDR syndrome (hypoparathyroidism, sensorineural deafness, renal dysplasia, OMIM #146255) with underlying mutations in the transcription factor GATA3.

However, inappropriately low or normal levels of PTH may also point to an activating mutation in the *CASR* gene encoding the Ca^{2+} sensing-receptor (CaSR) or in the *GNA11* gene encoding the accessory G-protein G-alpha-11 causing autosomal-dominant hypocalcemia type 1 and 2, respectively (ADH1, OMIM #601198; ADH2, OMIM #615361). These entities will be discussed in more detail below. ADH1 might be differentiated from the abovementioned disorders by determination of urinary calcium excretion: In contrast to low urinary calcium excretions found in primary forms of parathyroid function, urinary calcium excretions are inappropriately high in face of hypocalcemia in patients with ADH1.

Elevated levels of PTH in the presence of a normal renal function are also found in acquired calcium- and vitamin D-deficient rickets. Whereas hypocalcemia might also be an associated finding in renal Fanconi syndrome, it is rather uncommon in other disorders of the proximal tubule such as hypophosphatemic rickets, Dent's disease, or Lowe syndrome.

A heterogeneous group of rare hereditary disorders leading to the common feature of PTH end-organ resistance and hypocalcemia despite elevated PTH serum levels is pseudohypoparathyroidism (PHP1A OMIM #103580; PHP1B, OMIM #612462; PHP1C, OMIM #203330). Different genetic defects have been discovered as the underlying cause including loss of function mutations in the *GNAS* gene encoding the Gs-alpha isoform or methylation defects of the *GNAS* gene locus [35].

The extracellular calcium-sensing receptor (CaSR) plays an essential role in calcium and magnesium homeostasis by influencing not only PTH secretion but also by directly regulating the rate of calcium and magnesium reabsorption in the kidney. It was first cloned by Brown and colleagues in 1993 [36]. Along the distal nephron, the CaSR is expressed basolaterally in TAL and DCT as well as at both the apical and basolateral membrane of the collecting duct [37]. CaSR activation leads to coordinate changes in renal calcium and magnesium excretion and in water diuresis [38]. Dilution of the urine by decreasing aquaporin expression in the collecting duct is thought to minimize the risk of stone formation in the face of an increase in calcium and magnesium excretion.

The CaSR is a dimeric cell-surface protein belonging to the diverse group of G-protein coupled receptors. It has a large extracellular domain that was crystallized and shown to adapt a venus flytrap (VFT) conformation binding extracellular calcium at multiple sites [39]. Interestingly, recent data indicate additional anion binding sites through which phosphate is able to inhibit the CaSR in a noncompetitive antagonistic fashion and thereby induce PTH secretion from the parathyroid gland [40].

Via interaction with its transmembrane and intracellular domains, the CaSR acts through an intracellular signaling primarily mediated by the G proteins G_q and G_{11} [19, 41]. These activate phospholipase C resulting in the production of inositol 1,4,5 triphosphate and diacylglycerol (DAG). Downstream intracellular events finally result in a decrease in PTH release from the parathyroid gland and in an inhibition of renal tubular calcium absorption.

Calcium sensing is also influenced by the level of CaSR surface expression. The CaSR is internalized from the plasma membrane by clathrin-mediated endocytosis. A central component of these clathrin-coated vesicles (CCVs) is the adaptor protein 2 (AP2) that forms a heterotetrameric complexes of α, β, μ, and σ (AP2S1) subunits [42]. Several diseases associated with both activating and inactivating mutations in the *CASR* gene, *GNA11*, and *AP2S1* have been described.

Autosomal Dominant Hypocalcemia

Autosomal dominant hypocalcemia 1 (ADH1, OMIM #601198) is caused by activating mutations in the *CASR* gene. Affected patients typically manifest during childhood with seizures or carpopedal spasms. Laboratory evaluation reveals the typical combination of hypocalcemia and low PTH levels. Serum calcium levels are typically in a range of 6 to 7 mg/dL. In addition, many patients also exhibit moderate hypomagnesemia [43, 44]. Patients are often given the incorrect diagnosis of primary hypoparathyroidism on the basis of inappropriately low PTH levels. As indicated above, the differential diagnosis can be established by determination of urinary calcium excretions which are low in primary hypoparathyroidism, whereas ADH1 patients are thought to exhibit increased renal calcium excretions. The differentiation of ADH from primary hypoparathyroidism is of particular importance because treatment with active vitamin D in ADH may result in a dramatic increase in hypercalciuria and the occurrence of nephrocalcinosis and impairment of renal function. Therefore, therapy with active vitamin D or calcium supplementation should be reserved for symptomatic patients with the aim to maintain serum calcium levels just sufficient for the relief of symptoms [44].

Activating *CASR* mutations lead to a lower setpoint of the receptor or an increased affinity for extracellular calcium and magnesium. This inadequate activation by physiological extracellular calcium and magnesium levels results in diminished PTH secretion in the parathyroid gland as well as a decreased reabsorption of both divalent cations mainly in the cTAL of the kidney tubule. A severe degree of hypocalcemia and hypomagnesemia is observed in patients with complete activation of the CaSR at physiologic serum calcium and magnesium concentrations who may also exhibit a Bartter-like phenotype [45]. In these patients, CaSR activation inhibits TAL-mediated salt and divalent cation reabsorption to an extent that cannot be compensated in later nephron segments [45].

Genetic heterogeneity in autosomal dominant hypocalcemia (ADH2, OMIM #615361) was demonstrated in 2013 by discovery of heterozygous missense mutations in *GNA11* encoding the accessory G-protein $G_{\alpha 11}$ of the CaSR [46, 47]. The identified variants were shown to increase CaSR signaling and inositol 1,4,5-triphosphate synthesis resulting in a leftward-shift of the concentration-response curve with significantly lower EC_{50} values for extracellular Ca^{2+} [46].

Hypercalcemia

In contrast to hypocalcemia, hypercalcemia represents a rather uncommon finding in infancy and childhood but requires prompt diagnostic workup and targeted therapy [48, 49]. Hypercalcemia is defined as ionized Ca^{2+} levels above 1.35 mmol/L, usually with a concomitant elevation of total serum calcium levels above 2.7 mmol/L. While most children with a mild degree of hypercalcemia remain asymptomatic, more severe hypercalcemia can lead to a serious clinical disease pattern that may even be life-threatening. Symptoms of hypercalcemia include failure to thrive, weight loss, dehydration, fever, muscular hypotonia, constipation, irritability, lethargy, and disturbed consciousness. Cardiovascular findings may comprise bradycardia, shortened QT interval, and arterial hypertension (Table 2).

An impairment of renal function by volume contraction can further limit renal calcium elimination and therefore aggravate the excess in serum calcium levels. On the other hand, the increase of renal calcium excretion in hypercalcemic conditions is a risk factor for the development of nephrocalcinosis and stone formation.

The assessment of the patient`s past medical history should comprise medications including over-the-counter vitamin preparations and family history with a focus on disturbances in calcium metabolism, renal disease, and urolithiasis.

The etiology of hypercalcemia in children and especially infants significantly differs from that in adulthood where primary hyperparathyroidism and malignancy represent the most common underlying causes [50]. In early life, the time at which symptoms occur may yield an important information on the underlying etiology. Especially in infants, rare hereditary disorders should be considered in the differential diagnosis [48].

Table 2 Symptoms of acute hypercalcemia

Neurology	
	Muscle weakness
	Irritability/confusion
	Somnolence, stupor, coma
	Abnormal behavior
	Headaches
Gastrointestinal tract	
	Loss of appetite
	Nausea/vomiting
	Constipation
	Abdominal cramping
	Pancreatitis
Kidneys and urinary tract	
	Polyuria/polydipsia
	Dehydratation
	Nephrocalcinosis
	Nephro-/urolithiasis
Musculoskeletal system	
	Bone pain
	Ectopic calcifications
Heart/cardiovascular system	
	Shortened QT-interval
	Cardiac arrhythmia
	Arterial hypertension

The diagnostic work-up, next to a thorough medical history, requires comprehensive laboratory testing including molecular genetics. Next to the parallel measurement of serum and urine electrolytes, the determination of PTH and vitamin D metabolites represents the most important element. Potential diagnostic parameters are summarized in Table 3.

Inappropriately normal or elevated levels of PTH point to a primary defect in the parathyroid gland. Next to primary hyperparathyroidism which is extremely rare in infancy and childhood, hypercalcemia caused by elevated PTH might be caused by hereditary disorders of the CaSR or associated proteins. In contrast to dominant activating CaSR mutations as present in ADH1 (see above), inactivating CaSR mutations may be present in either heterozygous or homozygous/compound-heterozygous state leading to Familial Hypocalciuric Hypercalcemia 1 (FHH1) and Neonatal Severe Hyperparathyroidism (NSHPT),

Table 3 Evaluation of hypercalcemia in children

Laboratory tests	
Blood	Ionized and total calcium
	Sodium, potassium, chloride, magnesium, and phosphate
	Retention parameters
	Alkaline phosphatase
	Blood gases
	Intact parathyroid hormone (iPTH)
	Vitamin D metabolites (25-OH-D_3, 1,25-$(OH)_2$-D_3)
Optional	PTH-related peptide (PTHrP)
	Vitamin A
	Angiotensin converting enzyme (ACE)
	FISH (Williams-Beuren syndrome)
Urine	Calcium
	Creatinine
	-> calculation of Ca/creatinine ratio and tubular phosphate reabsorption
	Sodium, potassium, chloride, magnesium, and phosphate
Imaging studies	
	Ultrasound examination of kidneys and urinary tract
Optional	X-ray studies (long bones, thorax)
	Ultrasound of parathyroid glands
	Scintigraphy of parathyroid glands

respectively (see below). Genetic heterogeneity in FHH was demonstrated in 2013 by discovery of heterozygous mutations in *GNA11* (FHH2) and *AP2S1* (FHH3) [42, 46].

In face of an appropriate suppression of PTH, vitamin D metabolites should be evaluated. The determination of 25-OH-vitamin D_3 primarily helps to exclude overt vitamin D intoxication. While cut-off values vary in the literature, usually levels above 200 ng/mL are regarded as toxic [51]. Besides, serum levels of active 1,25-$(OH)_2$-vitamin D_3 are of critical importance for further diagnostic considerations. Elevated levels are not only observed in vitamin D poisoning, but also in disorders with extra-renal expression of 1-α-hydroxylase including granulomatous disease or subcutaneous fat necrosis. In these disorders, 1α-hydroxylase (CYP27B1) is expressed by monocytes and is not regulated by serum calcium, phosphate, PTH, and 1,25-$(OH)_2$-vitamin D_3, as in the kidney.

Acute therapeutic measures in hypercalcemic patients primarily aim at the lowering of serum calcium levels into the reference range resulting in a rapid relief of clinical symptoms. For this purpose, different pharmacologic approaches have been considered (Table 4).

Table 4 Therapy of hypercalcemia in children

General measures
Stop of vitamin D supplements
Calcium restriction in enteral and parenteral nutrition
Specific measures
Promotion of renal calcium excretion Furosemide 0.5–1 mg per kg body weight q6h
Inhibition of enteral calcium absorption/inhibition of vitamin D-conversion Glucocorticoids, that is, methylprednisolone ~1 mg per kg body weight q6h Sodium cellulose phosphate
Inhibition of calcium release from bone Bisphosphonates, that is, pamidronate 0.5–1 mg per kg body weight over 4–6h Calcitonin 4–8 IU/kg body weight
Inhibition of vitamin D-activation by 1α-hydroxylase Imidazole derivates, that is, ketoconazole 3–9 mg per kg body weight per day
Hemodialysis/hemofiltration

Before clarification of the underlying etiology, vitamin D supplements need to be stopped and, if adequate, a low-calcium diet should be implemented. A central component of every therapeutic strategy is a vigorous rehydration, usually performed via the intravenous route. Fluid amounts up to twice the daily requirements have been proposed. The pharmacologic treatment includes therapeutic measures to decrease calcium absorption from the intestine, to promote renal calcium excretion, as well as to inhibit calcium release from bone (Table 4). To increase renal calcium elimination, loop diuretics such as furosemide are widely used. Furosemide inhibits passive paracellular calcium reabsorption in the thick ascending limb by blocking active transcellular salt reabsorption. A fast and effective approach to inhibit enteral calcium absorption is the administration of glucocorticoids, that is, prednisolone. Next to this intestinal effect, glucocorticoids also inhibit the conversion of 25-OH-vitamin D_3 into active 1,25-$(OH)_2$-vitamin D_3, an effect that is of special importance in vitamin D-mediated hypercalcemia. Sodium cellulose phosphate (SCP) is a nonabsorbable cation exchange resin used for the removal of excess calcium from the body [52]. It was initially used in patients with so-called absorptive hypercalciuria in order to decrease renal calcium excretions and prevent stone formation [53]. In the acute phase of hypercalcemia, calcitonin may be used because of its prompt and pronounced effect on serum calcium levels; however, its therapeutic usefulness is limited by its short duration of action due to the development of end organ resistance. In the presence of intermediate to severe symptomatic hypercalcemia, bisphosphonates such as pamidronate are used [54]. If bisphosphonate therapy is considered, it is important to have the diagnostic work-up completed in advance to avoid misinterpretation of diagnostic tests for PTH and vitamin D metabolites. A class of drugs that are specifically used in vitamin D-mediated hypercalcemia are imidazole derivates such as ketoconazole [55]. Next to their antifungal effect, they also inhibit mammalian cytochrome P450 enzymes. Via inhibition of 1α-hydroxylase

(CYP27B1), they are able to effectively lower serum levels of active 1,25-$(OH)_2$-vitamin D_3 and consecutively normalize serum calcium levels. In single patients with extremely high serum calcium levels, hemofiltration and hemodialysis have been performed with success [56].

Hypercalcemia with Inappropriately High PTH

Familial hypocalciuric hypercalcemia (FHH; OMIM #145980, #145981, #600740)/Neonatal severe hyperparathyroidism (OMIM #239200)

Familial hypocalciuric hypercalcemia 1 (FHH1) as well as neonatal severe hyperparathyroidism (NSHPT) results from inactivating mutations of the CaSR present in either mono-allelic or bi-allelic state, respectively [57]. Genetic heterogeneity has been described in FHH with heterozygous variants in *GNA11* (FHH2) encoding accessory G-protein $G_{\alpha 11}$ of the CaSR and in *AP2S1* (FHH3) encoding adaptor protein $\sigma 1$ involved in CaSR recycling from the plasma membrane [42, 46]. Therefore, all three subtypes of FHH share the pathophysiology of a decreased CaSR-mediated calcium sensing. FHH patients normally present with mild to moderate hypercalcemia, accompanied by few if any symptoms and often do not require treatment. However, the occurrence of pancreatitis and chondrocalcinosis has been described in FHH patients [58]. Urinary excretion rates for calcium and magnesium are markedly reduced and serum PTH levels are inappropriately high. In addition, affected individuals also show mild hypermagnesemia [59].

In contrast to FHH, patients with NSHPT with two mutant *CASR* alleles usually present in early infancy with polyuria and dehydration due to severe symptomatic hypercalcemia. If unrecognized and not treated, hyperparathyroidism and hypercalcemia result in skeletal deformities, extraosseous calcifications, and severe neurodevelopmental deficit. Early treatment with partial-to-total parathyroidectomy therefore seems to be essential to prevent a negative outcome [60]. Serum phosphate levels are typically low due to increased PTH levels [61].

Hypercalcemia with Suppressed PTH and Inappropriately High 1,25-$(OH)_2$-Vitamin D_3

Idiopathic infantile hypercalcemia (OMIM #143880, #616963)

Idiopathic Infantile hypercalcemia (IIH) was first described in the 1950s after an epidemic occurrence in the United Kingdom (UK) [62, 63]. Affected infants clinically present with typical symptoms of severe hypercalcemia. Concomitant hypercalciuria typically leads to the development of early nephrocalcinosis. The laboratory analysis reveals serum calcium levels up to 5 mmol/L. Intact PTH is suppressed while levels of active 1,25-$(OH)_2$-vitamin D_3 are usually elevated or in the upper normal range. Early, a connection to exogenous vitamin D was suspected [64–66]. Some children exhibited a complex phenotype that became later known as the Williams Beuren syndrome [67, 68]. However, most hypercalcemic infants did not have syndromic features and were considered to be affected by a milder variant of the syndrome that was termed idiopathic infantile hypercalcemia (IIH) [62, 63].

The pathophysiology of IIH remained unknown until the discovery of loss-of-function mutations in the vitamin D catabolizing enzyme 25-OH-vitamin D_3-24-hydroxylase (CYP24A1) [69]. CYP24A1 is responsible for several sequential degradation steps that convert active 1,25-$(OH)_2$-vitamin D_3 into water soluble calcitroic acid [70, 71]. Loss-of-function mutations in the *CYP24A1* gene lead to an accumulation and increased action of active 1,25-$(OH)_2$-vitamin D_3. Functional studies *in vitro* demonstrated a complete loss of 24-hydroxylase enzyme function for most of the identified *CYP24A1* mutations [69]. In addition to the genetic analysis of the *CYP24A1* gene, the determination of 24-hydroxylated vitamin D metabolites by liquid chromatography/mass spectrometry (LC/MS) represents a quick and reliable test in the diagnosis of the carriers of two mutant *CYP24A1* alleles [72].

The critical role of a cumulative dose of exogenous vitamin D is underscored by the following observations. Under the most commonly used dose of 500 IU vitamin D_3 per day, symptoms usually develop after several months, most likely with an incomplete penetrance of the genetic trait. Higher doses of supplemental vitamin D most likely lead to an increased incidence of the disease in infancy and an earlier manifestation. Regimens using oral bolus doses of up to 600.000 IU cause symptoms of acute vitamin D toxicity in infants with CYP24A1 deficiency, while they are tolerated well by healthy individuals [73]. Finally, omitting vitamin D supplementation in a genetically affected infant due to symptomatic disease in the older sibling can prevent hypercalcemic episodes and the development of nephrocalcinosis [69].

After diagnosis of symptomatic hypercalcemia with inappropriately high levels of 1,25-$(OH)_2$-vitamin D_3, vitamin D prophylaxis is usually stopped and a low-calcium diet might be implemented. The restriction of dietary calcium has to be carefully monitored as it might lead to a defective mineralization of bones and may increase intestinal absorption of oxalates with a subsequent risk of stone formation. Next to these therapeutic measures, vigorous intravenous rehydration and strategies to reduce serum calcium levels as described above are used in symptomatic patients (Table 4).

Currently, it remains unclear why many affected individuals requiring acute treatment during infancy do not show recurrency of symptomatic hypercalcemia later in life. Potentially, compensatory mechanisms, that is, a down-regulation of 1α-hydroxylase (CYP27B1), are able to prevent excessive activation of vitamin D. Laboratory parameters reflecting disease activity, that is, a suppressed PTH and inappropriately high levels of 1,25-$(OH)_2$-vitamin D_3, are detectable for a long time during follow-up. Nephrocalcinosis persists; however, the impact on renal function deterioration is unclear. Interestingly, since the initial description of C*YP24A1* mutations, several groups were able to identify *CYP24A1* mutations also in adult patients with mild hypercalcemia, nephrocalcinosis, and recurrent kidney stone disease [74–76]. Therefore, compound-heterozygous/homozygous or only heterozygous mutations in the *CYP24A1* gene may represent a risk factor for the development of wider spectrum of diseases associated with an increased action of vitamin D.

Genetic heterogeneity in IIH was demonstrated by discovery of recessive mutations in *SLC34A1* encoding proximal-tubular sodium-phosphate co-transporter NaPi-IIa [77]. Patients with NaPi-IIa defects share phenotypic and biochemical features of patients with *CYP24A1* mutations though hypercalcemia is usually milder. In addition, they exhibit phosphate depletion and hypophosphatemia. The pathophysiology involves primary renal phosphate wasting, phosphate depletion, and suppression of the phosphaturic hormone FGF-23. FGF-23, next to its role in proximal-tubular phosphate reabsorption, negatively regulates vitamin D metabolism by inhibiting 1α-hydroxylase (CYP27B1) and promoting the degradation of active 1,25-$(OH)_2$-vitamin D_3 by 24-hydroxylase (CYP24A1) [77].

In addition to acute therapeutic measures also applied in patients with CYP24A1 defects, hypercalcemic patients with NaPi-IIa defects require phosphate supplementation in order to control calcium metabolism.

A significant subset of patients with NaPi-IIa mutations may also be diagnosed with isolated early nephrocalcinosis or present later in childhood or adolescence with nephrolithiasis [78]. Of note, prenatal renal calcifications secondary to *SLC34A1* mutations have been described and should be included in the differential diagnosis of fetal hyperechogenic kidneys [79].

Very similar biochemical changes as in NaPi-IIa deficiency have also been reported in patients with *SLC34A3* defects encoding the closely related proximal-tubular sodium-phosphate co-transporter NaPi-IIc [80]. Mutations in NaPi-IIc had initially been described in patients with hypophosphatemic rickets with hypercalciuria (HHRH) [81, 82]. Clinical manifestations related to hypercalcemia in infancy have not been reported yet in patients with *SLC34A3* defects. Patients with bi-allelic, but also mono-allelic *SLC34A3* mutations commonly present with nephrocalcinosis and/or kidney stones later in life [80].

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Inherited Disorders of Water Handling

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Abstract

Under normal circumstances, about 90% of the 180 L/day glomerular filtrate is constitutively reabsorbed in the proximal tubule and descending limb of Henle's loop. According to the needs, the remaining 10% of the fluid is reabsorbed in the collecting duct by a tightly regulated process under control of arginine vasopressin (AVP). After binding of AVP to arginine vasopressin type 2 receptors (AVPR2) in the basolateral membrane of collecting duct cells, aquaporin-2 (AQP2) water channels are inserted into the luminal membrane of these cells, allowing water reabsorption and urine concentration. Disorders of water handling are characterized by disturbances of this AVP-regulated system. In congenital nephrogenic diabetes insipidus (NDI), the kidney cannot concentrate urine in response to AVP, as a result of loss-of-function mutations in genes encoding AVPR2 and AQP2, resulting in polyuria and polydipsia. In recent years, extensive research has led to increased understanding of the cellular defects in NDI, with important implications for future development of targeted treatment of the disorder, with hope for better outcomes in comparison to the conventional symptomatic therapy. The very rare nephrogenic syndrome of inappropriate antidiuresis (NSIAD), caused by gain-of-function mutations in the gene encoding AVPR2, is the mirror image of NDI. In this disorder, urinary dilution is impaired, independent of the presence or absence of AVP. In this chapter, the focus will be on the physiology of water handling in the collecting duct and on its disturbances in congenital NDI. The clinical details, differential diagnosis, genetics, and conventional and possible future therapies of NDI will be discussed in detail.

Keywords

Congenital nephrogenic diabetes insipidus · Arginine vasopressin · Anticipatory thirst · Polyuria · Arginine vasopressin type 2 receptor · AQP2 water channel · X-linked · Autosomal · Nephrogenic syndrome of inappropriate antidiuresis · Acquired nephrogenic diabetes insipidus

Introduction

Disorders of water handling in the kidney lead either to dehydration or to overhydration. The vast majority of these disorders are acquired. The inherited disorders of water handling comprise congenital nephrogenic diabetes insipidus, which is associated with dehydration, and its mirror image, the nephrogenic syndrome of inappropriate antidiuresis, associated with overhydration and the risk of hyponatremia. In this chapter, after a detailed discussion on the physiology of urine concentration, we will mainly focus on the clinical details, diagnostics, differential diagnosis, genetics, and treatment of congenital nephrogenic diabetes insipidus.

Physiology of Urine Concentration, Including Vasopressin-Regulated Water Permeability

The physiologic action of vasopressin on the renal collecting duct is one of the most intensively studied processes in the kidney. Arginine vasopressin (AVP, or antidiuretic hormone (ADH)) is synthesized on the ribosomes of the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus as a large biologically inactive bound form. Within storage granules, the hormone is cleaved into the biologically active form and transported down the neuronal axons to the posterior pituitary and stored there. Following appropriate stimuli, AVP is secreted from the posterior pituitary to the circulation as biologically active hormone. AVP release is regulated by changes in plasma osmolality (by >2%) but can also occur in response to non-osmotic stimuli. These non-osmotic stimuli are generally related to changes in either total blood volume or the distribution of extracellular fluid. Patients with depleted effective circulating volume may secrete AVP even in the presence of low plasma osmolality. In addition, hypoxia, physical pain, emotional stress, and certain drugs (e.g., nicotine) influence the release of AVP.

There are also vasopressin-expressing neurons in the suprachiasmatic nucleus (SCN), and the circadian regulation of thirst and arginine vasopressin secretion protects against overnight dehydration. This is called anticipatory thirst. SCN vasopressin neurons project to thirst neurons in the organum vasculosum of the lamina terminalis (OVLT), where vasopressin is released as a neurotransmitter [36]. For example, the SCN opposes overnight adipsia by driving water intake before sleep and by driving the secretion of AVP and lowering body temperature to reduce water loss during sleep [37]. The anticipatory signals regulating thirst, sodium appetite, and hunger have now been described in experimental animals with optogenetic tools [4]. Thirst and AVP release are regulated by the classical homeostatic, interosensory plasma osmolality negative feedback as well as by novel, exterosensory, anticipatory signals. Decreases in blood vasopressin levels and thirst have been observed within minutes following water consumption, preceding and hence “anticipating” changes in plasma osmolality. These anticipatory signals for thirst and vasopressin release concentrate on the same homeostatic neurons and circumventricular organs monitoring the composition of blood. The lamina terminalis which is the median portion of the rostral wall of the third ventricle of the cerebrum is composed of three structures: the subfornical organ (SFO), the organum vasculosum of the lamina terminalis (OVLT), and the median preoptic nucleus (MnPO) (Fig. 1). Of these, SFO and OVLT lack the normal blood-brain barrier and, therefore, have direct access to circulating components including angiotensin II. The anticipatory signals from the oropharynx and gut are now well described: MnPO inhibitory neurons that coexpress glucagon-like peptide 1 receptor ($\mathrm{MnPO}^{\mathrm{GLP1r}}$) transmit oropharyngeal-induced satiation and send monosynaptic inhibition to $\mathrm{SFO}^{\mathrm{nNOS}}$ neurons (Fig. 1) [3]. Sensory stimuli from the oropharyngeal area are generally transported through cranial nerves V, VII, IX, and X to the central pattern generator within the nucleus tractus solitarius (NTS), which elicits swallowing action.

In its effector organ, the kidney, AVP binds to arginine vasopressin type 2 receptors (AVPR2) on the basolateral membrane of the principal inner medullary collecting duct cells and of the arcade cells. The arcades are long, highly branched renal tubule segments that connect distal convoluted tubules of several deep and mid-cortical nephrons to the origin of cortical collecting ducts. Upon binding of AVP, AVPR2 is activated and then stimulates GTP loading of the small GTPase αG_S – subunit of its coupled trimeric G-protein, eventually leading to dissociation of the G-protein

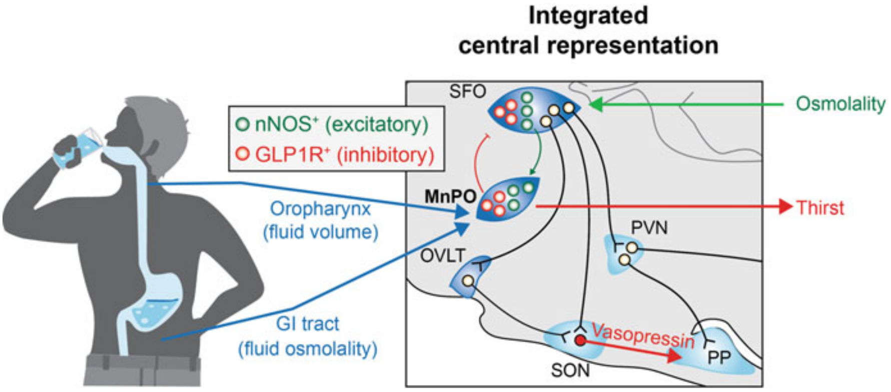

Fig. 1 **Anticipation for thirst and vasopressin regulation**. Following the detection of water in the mouth and its swallowing, there is a gut-to-brain signal: the osmolarity of the ingested fluid is perceived early in the digestive tract, probably in the small intestine or hepatic portal circulation, relayed by the vagus nerve and inhibits SFO^{Nos1} neurons well before any change in blood osmolality perceived by osmoreceptor cells. $MnPO^{GLP1R} \rightarrow SFO^{nNOS}$ forms an inhibitory circuit where $MnPO^{GLP1R}$ neurons are sensing volume in the oropharyngeal area and osmolarity in the gastrointestinal tract (represented schematically by blue arrows; modified from [126]). There is an integrated central representation of fluid balance at the level of individual MnPO neurons, which use this information to dynamically control drinking behavior and vasopressin secretion in real time

from the receptor (Fig. 2). GTP-αG_S can then bind to the membrane-associated adenylate cyclase 6 (AC6), activating it, primarily by stabilizing a catalytically competent form of the enzyme, which results in an increase in intracellular cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) [118]. The elevated cAMP levels stimulate protein kinase A (PKA), leading to phosphorylation of AQP2 which in turn initiates a redistribution of aquaporin-2 (AQP2) water channels from intracellular vesicles to the apical plasma membrane, rendering this membrane water permeable. The increase in apical membrane permeability allows water to flow from the tubule lumen to the hypertonic medullary interstitium, via AQP2 in the apical membrane and via AQP3 and AQP4, constitutive water channels in the basolateral membrane. This then leads to the formation of concentrated urine. This process restores plasma osmolality and volume and is regulated by a negative feedback; upon restoration of the water balance, AVP release into the blood decreases; AQP2 is internalized, thus reducing water permeability of the collecting ducts and water reabsorption. AQP2 is retrieved to intracellular vesicles, resulting in either lysosomal degradation or intracellular storage and relocation to the plasma membrane upon restimulation with AVP. At the basolateral side of the collecting duct, AVP binding to AVPR2 can also stimulate mechanisms that lead to termination of signaling. Desensitization and internalization of AVPR2, regulated by β-arrestin, decrease the amount of receptors in the plasma membrane [87].

In recent years, our knowledge of the AQP2 dynamics in the cell has increased significantly. For further details, the reader is also referred to several excellent reviews on this subject (reviews in [11, 29, 47, 78, 80, 121]). AQP2 is one of the 13 members of the aquaporin family of water channels. After transcription, AQP2 is folded into its native monomeric conformation in the endoplasmic reticulum, and homotetramerization takes place [38]. The tetramers are forwarded to the Golgi apparatus, where two out of four monomers are complex N-glycosylated. These functional water channels are then stored in intracellular vesicles to be transported to the apical membrane [84].

AQP2 plays a key role in short-term regulation and long term-adaptation of collecting duct water permeability. Short-term regulation (within minutes) is mediated by PKA-dependent phosphorylation and intracellular translocation of AQP2 from intracellular vesicles to the apical

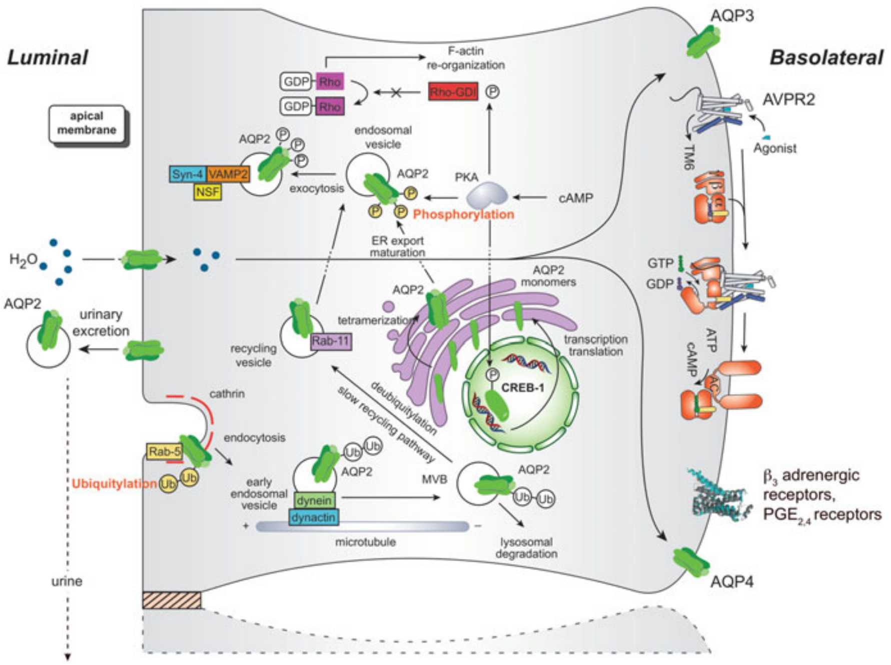
Luminal
apical membrane
Basolateral
AQP3
AVPR2
Agonist
TM6
F-actin re-organization
GDP Rho
GDP Rho
Rho-GDI
P
AQP2
endosomal vesicle
Syn-4
VAMP2
NSF
exocytosis
PKA
cAMP
Phosphorylation
ER export maturation
H_2O
AQP2
urinary excretion
AQP2 monomers
tetramerization
Rab-11
recycling vesicle
transcription translation
CREB-1
GTP
GDP
ATP
cAMP
AC6
cathrin
endocytosis
slow recycling pathway
deubiquitylation
Rab-5
Ubiquitylation
Ub
early endosomal vesicle
dynein
dynactin
MVB
microtubule
lysosomal degradation
β_3 adrenergic receptors,
$PGE_{2,4}$ receptors
AQP4
urine

Fig. 2 **Intracellular signal transduction pathway initiated by AVP binding to AVPR2**. Upon binding of AVP, AVPR2 is activated and then stimulates GTP loading of the small GTPase αG_S – subunit of its coupled trimeric G-protein, eventually leading to dissociation of the G-protein from the receptor. Following the publication of Kobilka and colleagues [39], we have represented here the hallmark of the G-protein-coupled receptor (GPCR) activation as the outward movement of the cytoplasmic end of transmembrane domain 6 (TM6) that opens up a large intracellular cavity to accommodate the G-alpha 5 subunit from G_s, leading to nucleotide exchange and activation of the G-protein. GTP-αG_S can then bind to the membrane-associated adenylate cyclase 6 (AC6), activating it. The resulting cAMP-production stimulation activates PKA, which in turn phosphorylates its target proteins AQP2, Rho-GDI, and CREB1. The transcription factor CREB-1-p stimulates AQP2 transcription, Rho-GDI-p initiates actin reorganization required for AQP2 transport, and AQP2-p homotetramers are transported to the apical membrane. Syn-4 and VAMP2 are involved in the insertion of AQP2 tetramers in the apical membrane. There, they render the membrane permeable for water, which is reabsorbed from the passing pro-urine and transported back into the blood stream by AQP3 and AQP4. Rab5-mediated AQP2 endocytosis by clathrin-coated vesicles is triggered by short-chain ubiquitylation and leads to termination of the response. Internalized AQP2 vesicles are transported to early and late endosomes as well as multivesicular bodies (MVBs) for storage. From MVBs, they can then either be lysosomally degraded or recycled via the Rab-11-dependent slow recycling pathway. The cAMP pathway can also be stimulated independent of AVPR2 signaling, for instance, by activation of ß3 adrenergic receptors or PGE2,4 receptors

plasma membrane. Long-term adaptation is seen when circulating AVP levels are increased over a period of hours and involves a change in the total abundance of the AQP2 protein in collecting duct cells as a result of regulatory actions at the transcriptional or posttranscriptional level. Both short- and long-term modulations of AQP2 are extremely complex and still not fully understood processes.

Short-Term Regulation of Water Reabsorption in Collecting Duct

PKA-dependent phosphorylation at the cytoplasmic carboxy-terminal serine 256 residue (pS256-AQP2) is essential and sufficient to cause trafficking of AQP2 to the apical membrane [34]. Phosphorylation at other canonical phosphorylation sites in the carboxy-terminus of AQP2 (i.e., Ser264, Ser269, Ser261) is involved also but apparently less critical (review in [29]). Phosphorylation at Ser269 inhibits AQP2 endocytosis, resulting in increased retention of AQP2 at the apical plasma membrane. Phosphorylation at Ser261 plays an important role in sorting AQP2 to degradation pathways. Studies using oocytes as a model system indicated that for plasma membrane localization, three out of four monomers in an AQP2 tetramer need to be phosphorylated [50]. PKA is the main kinase for AQP2 phosphorylation, but other kinases may potentially participate in the regulation of AQP2 trafficking. Besides PKA sites, putative phosphorylation sites for Protein kinase G (PKG), Protein kinase C (PKC), and casein kinase II are also present in the AQP2 sequence. In addition, it has been shown that anchoring of PKA to PKA-anchoring proteins (AKAPs), which ensures targeting of PKA to AQP2-bearing vesicles, is another prerequisite for AVP-mediated AQP2 translocation [57].

Reorganization of the actin cytoskeleton is also required for AQP2 transport to and accumulation in the apical membrane. The actin cytoskeleton most likely provides a network that anchors the AQP2-bearing vesicles in the unstimulated cell. Vasopressin has been shown to depolymerize apical F-actin in rat inner medullary collecting duct, resulting in the fusion of AQP2-carrying vesicles with the apical membrane [111], indicating that reorganization of the apical actin network may be critical in promoting the trafficking of AQP2-bearing vesicles. Activation of small GTPase RhoA induces F-actin-containing stress fibers and tonically inhibits AQP2 trafficking. Rho inhibition through PKA-mediated phosphorylation of Rho-GDP dissociation inhibitor (Rho-GDI) is shown to be a key event for actin reorganization inducing AQP2 translocation [58].

The plasma membrane insertion of AQP2-containing vesicles in the apical membrane is similar to the process of synaptic vesicle fusion with the presynaptic membrane and involves vesicle (v) soluble NSF attachment protein receptors (SNAREs) and target membrane (t) SNAREs. The apical membrane-specific t-SNARE is syntaxin 4 (Syn-4), which interacts specifically with the v-SNARE protein VAMP2 located on the cytoplasmic side of AQP2-containing endosomal vesicles [66, 71, 85]. V- and t-SNARES are recycled by the AAA-type ATPase NSF.

AQP2 is internalized after removal of AVP stimulation when the water balance is restored. During this endocytotic process, AQP2 accumulates in clathrin-coated pits and is internalized via a clathrin-mediated process [114]. Endocytosis is regulated by short-chain ubiquitylation at lysine 270 (K270) in the AQP2 terminal tail [51]. The process of ubiquitylation of AQP2 involves several ubiquitin E3 ligases, including NEDD4, and the heat-shock protein 70 (Hsp70)-interacting protein CHIP [76, 119, 122]. Specificity of the endocytotic AQP2 internalization is mediated by Rab5 protein, an effector-binding factor involved in plasma membrane to early endosome transport [90]. To be available for recycling, AQP2-containing endosomes need to be redistributed to the perinuclear region. This process is mediated by dynein-dependent transport along microtubules [73]. From the endosomal system – early/late endosomes and/or multivesicular bodies (MVBs) – AQP2 is either recycled by the Rab11-dependent slow recycling pathway or marked for lysosomal degradation [113]. Prolonged K270 ubiquitylation induces MVB trafficking and localization to internal vesicles of MVBs followed by lysosomal degradation, while deubiquitylation increases localization to early endosomes and the limiting membrane of MVBs and enables AQP2 recycling [51, 102, 113].

Long-Term Regulation of Water Reabsorption in Collecting Duct

Long-term adaptation of collecting duct water permeability following AVP stimulation is mediated by changing the expression of AQP2 mRNA and protein (review in [48]). An increase in cAMP levels result in PKA-mediated phosphorylation and activation of a cAMP-responsive element-binding protein 1 (CREB-1), which increases AQP2 expression by enhancing its gene transcription. Evidence is accumulating that nuclear receptor transcription factors (i.e., PPARγ, FXR, GR, MR, LXRs) are also involved in the long-term regulation of water transport in renal collecting ducts by either up- or downregulating AQP2 gene transcription and expression (review in [29, 125]). In addition, micro-RNAs (i.e., miR-32 and miR-137) appear to be important posttranscriptional regulators of AQP2 protein abundance [53, 96].

Alternative Signaling Pathways

The AVP-dependent cAMP-PKA pathway is the canonical pathway to regulate AQP2 expression and trafficking. In recent years, it has been demonstrated that alternative pathways bypassing AVPR2 signaling may also influence AQP2 regulation and trafficking (reviews in [48, 78]). These alternative pathways are interesting since they may be targets for therapy of NDI. The cAMP pathway can be stimulated by the activation of other G-protein-coupled receptors (GPCRs), such as the prostaglandin receptors (EP2/EP4), the calcitonin receptor, the secretin receptor, and the β3-adrenergic receptor (reviews in [48, 78]). In addition, AQP2 trafficking and abundance are affected by the cAMP-exchange protein directly activated by cAMP (Epac) pathway. The latter pathway is suggested to be involved in the long-term regulation of AQP2, whereas the cAMP-PKA pathway has independent short-term effects. The AVPR2-elicited pathway can also be bypassed in a cAMP-independent way. For instance, activation the Cyclic guanosine monophosphate (cGMP) pathway by nitric oxide donors or atrial natriuretic peptide promotes AQP2 trafficking and membrane insertion, and activation of the Wnt5-frizzled receptor promotes AVP-independent AQP2 phosphorylation via intracellular calcium/calmodulin/calcineurin signaling [2].

Congenital Nephrogenic Diabetes Insipidus

Congenital nephrogenic diabetes insipidus (NDI) is a rare inherited disorder, characterized by insensitivity of the distal nephron to the antidiuretic effects of AVP. As a consequence, the kidney loses its ability to concentrate urine, which may lead to severe dehydration and electrolyte imbalance (hypernatremia and hyperchloremia).

Clinical Presentation

Patients with NDI have normal birth weight, and pregnancies are not complicated by polyhydramnios. The urine-concentrating defect in NDI is present from birth, and manifestations of the disorder generally emerge within the first weeks of life. With breast milk feedings, infants usually thrive and do not develop signs of dehydration. This is because human milk has a low salt and protein content and therefore a low renal osmole load. With cows' milk formula feedings, the osmole load to the kidney increases, resulting in an increased demand for free water. This is usually not provided by oral feeding, and therefore, hypernatremic dehydration appears. Polyuria and polydipsia, irritability, poor feeding, recurrent vomiting, and poor weight gain are usually the initial symptoms [21, 107]. Patients are eager to suck but may vomit during or shortly after the feeding. Dehydration is evidenced by dryness of the skin, loss of normal skin turgor, recessed eyeballs, increased periorbital folding, depression of the anterior fontanel, and a scaphoid abdomen. Intermittent high fever is a common complication of the dehydrated state, predominantly in very young children. Body temperature can be normalized by rehydration. Seizures can occur but are rare and most often seen during therapy, particularly if rehydration proceeds too rapidly. Constipation is a common symptom in children with NDI. Nocturia and nocturnal enuresis are common complaints later in childhood.

If untreated, most patients fail to grow normally. In a retrospective study of 30 male NDI patients, most children grew below the 50th percentile, most of them having standard deviation (SD) scores lower than −1 [68]. Some well-treated patients, however, may achieve normal adult height. Catch-up growth occurs at least in some patients after normalization of water and electrolyte balance, especially in those with adherence to treatment. Bone maturation is generally not delayed.

In a more recent European report on the long-term follow-up of patients with NDI, growth was improved compared with historic cohorts with median height SDS being −1.1 at presentation and − 0.9 SD at last follow-up and median weight SDS being −2.1 and 0.2, respectively [107]. Initial feeding problems and the ingestion of large amounts of low-calorie fluid resulting in a decreased appetite may play roles in failure to thrive seen in NDI. Furthermore, it is possible that repeated episodes of dehydration have some as yet undetermined negative effects on growth.

Intellectual disability has long been considered an important complication of untreated NDI and assumed to be a sequel of recurrent episodes of severe brain dehydration and cerebral edema caused by overzealous attempts at rehydration. Additional evidence underscoring the assumption that NDI has adverse effects on the cerebrum is provided by several reports describing intracranial calcifications in NDI patients [109]. Such lesions are generally considered to be the result of hemorrhage or necrosis. Most of the reported patients with cerebral calcifications were intellectually disabled. Nowadays, intellectual disability is rare due to earlier recognition and treatment of NDI. Exact estimates of the current frequency of intellectual disability under modern treatment are unknown. One psychometric study reported 2 of the 17 male NDI patients (aged 3–30 years) have a total intelligence quotient of more than 2 SD below the norm. Fourteen patients had an intelligence score within or above the normal range, and one patient had a general index score between −1 and − 2 SD [40].

The psychological development of NDI patients is influenced by a persistent desire for drinking and the need for frequent voiding, which compete with playing and learning. Therefore, many NDI patients are characterized by hyperactivity, distractibility, short attention span, and restlessness. In the psychometric study mentioned earlier, the criteria for attention deficit hyperactivity disorder were met in 8 of 17 tested NDI patients [40].

In a recent cohort of NDI patients, one of 36 patients had severe global developmental delay which was already noted at his presentation at 7 months, and 5 of 28 children in whom information on learning and behavior information was available had attention deficit hyperactivity disorder (ADHD) and/or learning difficulties and/or impaired concentration, pointing to overall improvement in mental and psychological development of individuals with NDI over the last decades [21, 107].

Urological complications are noted in 40%–50% NDI patients [20, 107]. Persistent polyuria can result in the development of megacystis, trabeculated bladder wall, hydroureter, and hydronephrosis [21, 68, 107]. Urinary tract distension may be seen on ultrasound examination even in infants and young children. Potential complications of urinary tract dilatation are rupture of the urinary tract, infection, intractable pain, improper bladder function, and/or kidney failure. These complications may occur as early as the second decade of life. Large-capacity hypotonic bladder dysfunction might require clean intermittent catheterization.

Patients should be trained to void regularly in order to assure that maximal urinary bladder capacity remains within normal range. Both patient groups with *AVPR2* and *AQP2* mutations can develop urinary tract dilatation and bladder dysfunction. Nocturnal enuresis usually persists longer than in healthy children due to pronounced polyuria. One study reported the median age at resolution being around 11 (range 5–16) years [107].

Chronic kidney disease stage 2 or higher is reported in about 20% of the patients; however, no case of end-stage kidney disease has been reported so far [21, 107].

Diagnostic Procedures

The observation of polyuria in a dehydrated infant, together with the finding of a high serum sodium concentration and inappropriately dilute urine (Uosm/Posm), provides presumptive evidence for a renal concentrating defect. To confirm the concentrating defect and to distinguish the renal form of diabetes insipidus from the central form, a vasopressin test is performed with 1-deamino-8-D-arginine vasopressin (DDAVP), a synthetic analogue of the natural arginine vasopressin that produces a high and prolonged antidiuretic effect. In the test, DDAVP (10 μg for infants <1 year old, 20 μg for children >1 year old) is administered intranasally. Urine is collected during the subsequent 5.5 hours. The first collected portion of the urine should be discarded. The maximal urine osmolality in any collected aliquot is chosen as a measure of the concentrating capacity. After DDAVP administration, NDI patients are unable to increase urinary osmolality, which remains below 200 mOsm/kg H_2O (normal values: <1 year old >600, between 1 and 2 years old between 600 and 800, >2 years old >800 mOsm/kg H_2O) and cannot reduce urine volume or free-water clearance.

Performing a water restriction test is not recommended for the diagnosis of NDI as it can lead to pronounced dehydration and unneeded suffering of the patients.

Plasma vasopressin levels are normal or only slightly increased in affected children. Other laboratory findings have been described, which mainly result from chronic dehydration. Serum sodium concentration is generally elevated and may be above 170 mmol/L. There is also an increase in serum chloride concentration and retention of urea and creatinine. All values normalize by adequate rehydration. In addition, reduced glomerular filtration rate (GFR) and renal blood flow can return to normal when a normal hydration state has been achieved.

The primary congenital form of NDI has to be differentiated from central diabetes insipidus (due to lack of AVP) and from the secondary or acquired forms, which are much more common. In our experience, the urinary osmolality obtained after DDAVP administration in secondary disorders is always higher than in NDI.

Secondary Nephrogenic Diabetes Insipidus

In addition to primary forms of congenital NDI, a few cases of secondary inherited NDI have been reported [11] (Table 1). These patients have Mendelian diseases that affect tubular function with NDI as a secondary complication. In some such patients, the NDI symptoms dominate the clinical picture, leading to an initial misdiagnosis, with the true underlying cause of disease subsequently identified. Most of the primary diseases associated with secondary NDI, such as Bartter syndrome and apparent mineralocorticoid excess, are associated with hypokalemia and hypercalciuria. Patients with polyhydramnios, hypercalciuria, and isosthenuria have been found to bear *KCNJ1* (ROMK), *SLC12A1* (NKCC2), and *MAGED2* mutations. Patients with polyhydramnios, profound polyuria, hyponatremia, hypochloremia, metabolic alkalosis, and sensorineural deafness were found to bear *BSND* (Barttin) mutations. These studies demonstrate the critical importance of the proteins ROMK, NKCC2, and barttin in transferring NaCl to the medullary interstitium and thereby generating, together with urea, a hypertonic milieu.

Acquired Nephrogenic Diabetes Insipidus

Although the hereditary forms of NDI are relatively rare, a wide range of pathologic conditions and drug treatments can lead to acquired NDI (Table 1). In our experience, the urine osmolality obtained after DDAVP administration in these acquired disorders is always higher than in congenital NDI. All these disorders have been shown to coincide with decreased expression of AQP2 or deregulated AQP2 trafficking to the apical membrane [32, 63, 72, 105]. For instance, prolonged

Table 1 Causes of secondary or acquired nephrogenic diabetes insipidus

Monogenetic diseases associated with secondary NDI
Renal Fanconi syndromes
Bartter syndrome (type 1 or type 2)
Familial hypomagnesemia with hypercalciuria and nephrocalcinosis
Distal renal tubular acidosis (dRTA)
Apparent mineralocorticoid excess (AME)
Ciliopathies (nephronophthisis, Bardet-Biedl syndrome, etc.)
Other renal diseases
Obstructive uropathy
Renal dysplasia
Postischemic damage
Amyloidosis
Sarcoidosis
Chronic renal failure
Renal impairment in sickle cell disease or trait
Drug-induced
Lithium
Ifosfamide
Amphotericin B
Tetracyclines
Biochemical abnormalities
Hypercalcemia and hypercalciuria and nephrocalcinosis
Hypokalemia

treatment with lithium, the drug of choice for treating bipolar disorders and prescribed to 1 in 1000 of the population, results in the development of NDI in at least 20% of treated individuals [120]. The development of lithium-NDI is believed to occur in two phases. In the first short-time phase, lithium causes a decrease in AQP2 expression (review in [55]). Lithium enters the cells via epithelial sodium channel (ENaC) and accumulates in principal cells. How lithium downregulates AQP2 is not clear but likely involves glycogen synthase kinase 3 (GSK3)-β, which is important in AVP-regulated antidiuresis and is inhibited by lithium. GSK3 inhibitors other than lithium also reduce AQP2 expression in collecting duct cell cultures, and ablation of GSK alpha or beta in mice causes an inability to concentrate urine by dehydration or DDAVP [19]. Lithium also influences AQP2-mediated water reabsorption by increased tubular release of prostaglandin E2. In the second phase, lithium reduces the percentage of principal cells in the collecting duct to the advantage of intercalated cells, involved in acid-base homeostasis [18]. The exact contribution of this collecting duct remodeling in the lithium-induced resistance to vasopressin remains to be elucidated. Autophagic degradation of aquaporin-2 has been implicated in hypokalemia and hypercalcemia-induced NDI [19].

Nephrogenic Diabetes Insipidus: Genetics

Three different inheritance patterns of congenital NDI have been recognized. In most cases (about 90%), NDI is transmitted as an X-linked recessive trait (MIM304800). In these families, female carriers who are usually unaffected, transmit the disease to their sons, who display the complete clinical picture. In 1992, mutations in *AVPR2* were shown to underlie X-linked NDI [89, 103]. In a minority of families (about 10%), the transmission and phenotypic characteristics of NDI are not compatible with an X-linked trait. In these families, females display the complete clinical picture of NDI and are clinically undistinguishable from affected male family members. Family pedigrees suggested the existence of both an autosomal recessive (MIM 222000) and an autosomal dominant form (MIM 125800) of NDI. It was subsequently demonstrated that both autosomal forms of NDI are caused by mutations in *AQP2* [22, 81]. The prevalence of NDI is not exactly known, but the disease is assumed to be rare. The estimate of the prevalence of NDI in Quebec, Canada, is 8.8:1,000,000 males. However, owing to chance population genetic events, such as a founder effect, the incidence of NDI is elevated in certain regions, for example, in Utah and Nova Scotia [11].

X-Linked Nephrogenic Diabetes Insipidus: Mutations in AVPR2

The X-linked form of NDI is caused by loss-of-function mutations in *AVPR2* (MIM 300538) (reviews in [8, 11, 46, 59, 80, 121]). *AVPR2* is a relatively small gene, consisting of three exons separated by two short intervening sequences (introns); two isoforms are known that are generated by alternative splicing [30]. *AVPR2* is localized on the X chromosome on locus Xq28. The cDNA encodes a receptor protein of 371 amino acids, has a predicted molecular mass of approximately 40 kDa, and shares the general structure of a G-protein-coupled receptor consisting of seven hydrophobic transmembrane helices, connected by extracellular and intracellular loops. The receptor contains one unique consensus sequence site for N-linked glycosylation in the extracellular amino-terminus [42] and phosphorylation sites for G-protein-coupled receptor kinases (GRK) represented by a serine cluster in the carboxy-terminus [43]. The amino-terminal part of the protein including the first transmembrane domain and the positively charged first intracellular loop are important for proper insertion and orientation in the membrane [110]. A conserved glutamate-dileucine motif in the intracellular carboxy-terminal part of the receptor is essential for receptor transport from the endoplasmic reticulum (ER) to the Golgi apparatus [61]. Two conserved adjacent cysteines in the C-terminus are palmitoylated, thereby anchoring the carboxy-tail to the plasma membrane and controlling the tertiary structure of this region of the receptor [110].

To date, there are more than 280 distinct disease-causing mutations in *AVPR2*, and the number is constantly increasing (reviews in [7, 59, 80, 121], and www.hgmd.org). The mutations are not clustered in one domain of the AVPR2 but are scattered throughout the protein, except for the part coding for the N- and C-terminal tails of the receptor. More than 50% of the mutations are missense mutations; nonsense mutations (8%), splice site mutations (1%), small deletions (18%), small insertions/duplications (7%), small indels (2%), large gene deletions (8%), large insertions/duplications (1%), and complex rearrangements (2%) account for the remainder of mutations (reviews in [7, 46, 59, 80, 121], and www.hgmd.org). Several mutations (p.Asp85-Asn, p.Val88Met, p.Arg113Trp, p.Tyr128Ser, p.Arg137His, p.Ser167Leu, p.Arg181Cys, p.Arg202Cys, p.Ala294Pro, and p.Ser315Arg) are recurrent as evidenced by the fact that these mutations were found on different haplotypes in ancestrally independent families. The most frequent of these recurrent mutations (p.Asp85Asn, p.Val88Met, p.Arg113Trp, p.Arg137His, p.Ser167-Leu, p.Arg181Cys, p.Arg202Cys) occur at potential mutational hotspots [8].

AVPR2 mutations seem to be present in all ethnical groups tested with no preference of one mutation for any ethnic group over others. However, two founder mutations, the p.Trp71* "Hopewell" mutation in Ulster Scott immigrants and the p.Leu312* in a large Utah pedigree, result in an increased prevalence of X-linked congenital NDI in their descendants in certain communities in Nova Scotia, Canada, and Utah [33].

The molecular mechanism underlying the renal insensitivity for AVP differs between mutants. As upcoming pharmacological treatments for NDI likely depend on the underlying mechanism, GPCR mutations in general and *AVPR2* mutations in particular have been divided in five different classes according to their cellular fate ([98], review in [78]).

Class I comprises all mutations that interfere with proper transcription, mRNA processing, and translation, resulting in truncated proteins that are often rapidly degraded, such as splice site, frameshift, and nonsense mutations [98]. Class II mutations are missense or insertions/deletions of one or more nucleotide triplets, resulting in fully translated proteins. Due to the mutation, however, mutant receptors are misfolded and retained in the endoplasmic reticulum (ER), as the ER is the organelle that has the cellular quality control over proper folding and maturation of synthesized proteins. Misfolded proteins are subsequently mostly targeted for proteasomal degradation [26]. Intracellular entrapment of missense AVPR2 mutants and their rapid degradation likely represent the most important cause of NDI, as more than 50% of the *AVPR2* mutations are missense mutations and cellular expression revealed that most of these result in ER-retained proteins. Class III mutations result in plasma membrane-expressed receptors with reduced affinity for the stimulatory G_s protein. Mutations in this group are missense mutations and in-frame deletions, mostly located in transmembrane and intracellular domains [99]. Class IV mutations have low affinity for

AVP [99]. Finally, class V mutations are missorted to incorrect cellular compartments, like arrestin-positive endocytotic vesicles [5]. Sometimes, mutants do not exert a full phenotype of a particular class and then often also show features of another class. For example, some AVPR2 missense mutants are partially ER-retained (class II) but are also partially expressed in the plasma membrane, where they might show a reduced G-protein coupling (class III) or AVP binding (class IV). As such, it provides an explanation for the observed small antidiuretic response to high doses of DDAVP in NDI patients harboring such mutations [92].

Nephrogenic Syndrome of Inappropriate Antidiuresis

NSAID, with four identified *AVPR2* gain of function mutations: R137C, R137L, F229V, and I130N, is the mirror image of NDI. [7]. In NDI, the kidneys cannot concentrate the urine, whereas in NSIAD, urinary dilution is impaired, independent of the presence or absence of vasopressin. Consequently, patients with NDI are at risk of hypernatremic dehydration, whereas hyponatremia is a typical manifestation of NSIAD, mimicking the syndrome of inappropriate antidiuresis (SIADH); unlike SIADH, AVP levels are usually low or undetectable in NSIAD [28]. The diagnostic pathways also mirror: in NDI, an agonist for the AVPR2, such as synthetic V_2-vasopressin analogue 1-deamino-8-D-arginine vasopressin (DDAVP), is given to assess the ability of the kidneys to concentrate urine. Conversely, administration of an AVPR2 antagonist, such as tolvaptan, provides an assessment of urinary dilution capacity in patients suspected of NSIAD.

Recently, it was shown that NSIAD can also be caused by mutations in Gsα-coding *GNAS* gene. Miyado et al. reported two families with a dominantly inherited form of NSIAD segregating with the GNAS variants p.F68_G70del and p.M255V [79]. The *GNAS* mutation p.F376V was reported in two unrelated patients with hyponatremia, and it was associated with additional clinical symptoms, suggesting gain of function not only of AVPR2 but also of other GPCR, including the lutropin (LHGR) and parathyroid hormone (PTH1R) receptors [10]. The severity of the phenotype in the patients with the dominantly inherited mutations was quite variable: some patients presented with seizures in early childhood associated with euvolemic hyponatremia, inappropriately elevated urine osmolality, and suppressed vasopressin levels. Other patients had no apparent symptoms and were normonatremic when investigated, but they had elevated urine osmolality despite suppressed vasopressin levels and a history of spontaneously low fluid intake. Symptomatic family members were treated with fluid restriction with normalization of hyponatremia.

Genotype-Phenotype Correlations in X-Linked NDI?

Almost all pathogenic variants in *AVPR2* result in a uniform clinical NDI phenotype with polyuric manifestations in the first weeks of life and poor growth. There are, however, a few exceptions to this rule. Several mutations appear to be associated with a milder form of NDI, characterized by a later manifestation, not at birth but later in childhood, and without growth retardation. At present, 18 *AVPR2* pathogenic variants resulting in partial NDI have been reported (Table 2; review in [59]).

Intrafamilial variability of the X-linked NDI phenotype has also been described. A nice example is the case described by Kalenga et al., who reported a Belgian family in which the p.Arg137His pathogenic variant was associated with severe NDI in the proband but with very mild NDI in his affected brother [49]. Genetic and/or environmental modifying factors are likely to account for this intrafamilial phenotype variability.

The Autosomal Recessive and Autosomal Dominant Forms of Nephrogenic Diabetes Insipidus: Mutations in the Aquaporin-2 Water Channel

Both the autosomal recessive and the autosomal dominant types of NDI are caused by mutations in the *AQP2* water channel gene (MIM 107777;

Table 2 *AVPR2* pathogenic variants resulting in partial NDI

Pathogenic *AVPR2* variants	Effect of the variant(s)	References
p.Asn317Lys p.Asn317Ser p.Asn321Tyr p.Met311Val	Reach the cell surface with impaired ligand capacity and partial AVP/DDAVP binding	[83]
p.Asp85Asn	Decreased ligand-binding affinity and decreased coupling to G_s	[104]
p.Gly201Asn	Decreased number of cell surface AVPR2 receptors	[104]
p.Pro322Ser p.Val88Met	Reduced cell surface expression and/or decreased binding affinity for AVP	[1, 12]
p.Ser334del p.Tyr128Ser p.Thr273Met p.Ser329Arg	Impaired intracellular trafficking	[27, 70, 116]
p.Arg104Cys	Decreased AVP binding most likely due to conformational changes	[27]
p.Leu161Pro c.276A > G (splice site)	Unknown	[123], [108]

GenBank accession number z29491). Human *AQP2* is a small gene consisting of four exons, comprising 5 kb genomic DNA. The 15 kb mRNA encodes a protein of 271 amino acids which has a predicted molecular weight of 29 kDa [35]. AQP2 belongs to a family of integral membrane proteins, aquaporins, which function as selective water transporters throughout the plant and animal kingdom. In mammals, 13 different aquaporins have been identified to date, eight of which (aquaporins 1–4, 6–8, and 11) are highly expressed in the kidney. In the plasma membrane, AQP2 exists as a homotetramer, with each monomer containing a water pore. Each AQP2 monomer has six membranes spanning α-helical domains and intracellular N- and C-termini. The six transmembrane helices are connected via three intracellular loops (A, C, and E) and two extracellular loops (B and D), the latter containing highly conserved Asn-Pro-Ala (NPA) sequence motifs. The six transmembrane helices surround a single, narrow water-conducting channel, while the two extracellular loops fold into the membrane from opposite sites with their NPA motifs forming a constriction in the middle of the permeation channel. Another conserved structural feature, the aromatic/arginine (ar/R) constriction site located at the extracellular side of the channel, acts as a selectivity filter, preventing permeation of all molecules bigger than water ([31], review in [29]). AQP2 is exclusively localized in the apical membrane and a subapical compartment of collecting duct cells. It is upregulated by dehydration or AVP, indicating that it is the AVP-regulated water channel.

To date, 52 putative disease-causing mutations in *AQP2* have been identified in families with autosomal recessive NDI (reviews in [8, 59, 78, 121]). These include 42 missense mutations, two nonsense mutations, two small deletions, and four splice site variants. Most mutations are found between the first and last transmembrane domain of AQP2. Expression studies in *Xenopus laevis* oocytes have revealed that most *AQP2* missense mutations that cause recessive NDI are class II mutations. Thus, these mutations lead to misfolding of the mutant protein, retention in the endoplasmic reticulum (ER), and rapid degradation of *AQP2* (review in [121]). When overexpressed in oocytes and Chinese hamster (CHO) cells, six of these AQP2 mutants (A147T, T126M, G64R, L22V, A47V, and T125M) confer water permeability [74, 117]. This indicates that at high expression levels, these AQP2 mutant proteins escape from the ER and are routed to the plasma membrane, where they are functional. In terms of possible treatment strategies, these results are of high importance, since they suggest that functional channels may be stimulated to reach the plasma membrane by restoring mutant trafficking.

One AQP2 missense mutant, P262L, located in the AQP2 C-terminal tail, a region until then believed to result in dominant NDI, surprisingly was found to be involved in recessive NDI [23]. In cell biological experiments, it was shown that the P262L mutant is a functional water channel that forms hetero-oligomers with wt-AQP2. These wt-AQP2/AQP2-P262L heterotetramers are located in the apical membrane, indicating that the apical sorting of wild-type AQP2 is dominant over the missorting signal of AQP2-P262L. This is different from dominant NDI, because in this form mutants retain wt-AQP2 in intracellular locations (see below). The recessive inheritance in the two patients encountered (patients were heterozygous for a R187C or A190T mutation on one allele, combined with a P262L mutation on the other allele) can be explained as follows: AQP2-R187C and AQP2-A190T are retained in the ER and do not interact with AQP2-P262L. AQP2-P262L folds properly and assembles in homotetramers but will be retained mainly in intracellular vesicles. The consequent lack of sufficient AQP2 proteins in the apical membrane of the patients' collecting duct cells explains their NDI phenotype. In the parents coding for wt-AQP2 and AQP2-R187C or AQP2-A190T, wt-AQP2 will not interact with either mutant but will form homotetrameric complexes, of which the insertion into the apical membrane will be regulated properly by vasopressin and will give a healthy phenotype. In the parents coding for wt-AQP2 and AQP2-P262L, both proteins likely assemble into heterotetramers. The dominancy of wt-AQP2 sorting on the localization of AQP2-P262L will result in proper AVP-regulated trafficking of the heterotetrameric complexes to the apical membrane and will also give a healthy phenotype [23].

More than ten families have been described with autosomal dominant NDI, initially uncovered due to father-to-son transmission of the disease. In these families, subsequent sequencing of *AQP2* revealed putative disease-causing mutations in one *AQP2* allele. At least 13 pathogenic variants that give rise to autosomal dominant NDI have been described. These are six missense variants, one 1-bp insertion, and six small deletions [59, 78, 121]. All mutations causing dominant NDI are located in coding region of the C-terminal tail of AQP2, which is not part of the pore-forming segment, but contains important sorting signals that govern intracellular transport of the protein [99]. Indeed, all functionally tested mutant AQP2 proteins found in dominant NDI appeared to be folded functional water channels that were sorted to other subcellular locations in the cell than wt-AQP2, e.g., late endosomes/lysosomes and the basolateral membrane. Because none of these mutants were misfolded, they were, in contrast to AQP2 mutants in recessive NDI, able to interact and form heterotetramers with wt-AQP2. Due to this wt-mutant interaction and the dominancy of the missorting signals in the mutant protein, the wt-mutant complexes are also missorted. Formation of heterotetramers with wt-AQP2 has been shown for most of the dominant AQP2 mutants. For instance, expression studies in polarized cell lines have revealed the dominant AQP2- E258K mutant is routed to the Golgi complex or late endosomes/lysosomes [81]. In co-expression studies with wild-type AQP2, a dominant-negative effect was observed, caused by impaired routing of wild-type AQP2 to the plasma membrane after hetero-oligomerization with the E258K mutant [52]. Mistargeting to the basolateral membrane has been reported for the AQP2-721delG, AQP2–763-772del, AQP2–812-818del, and AQP2–779-780insA mutants [62, 75]. TheAQP2-727delG mutant was shown to interfere with the routing of wild-type AQP2 to the apical membrane by its mistargeting to the basolateral membrane and late endosomes/lysosomes [75]. The loss of appropriate AQP2 heterotetramer trafficking in dominant NDI is caused by several mechanisms. The phosphorylation site at Ser256, serving as an introducible apical sorting signal, may be inactivated, overruled by basolateral sorting signals, or reprogrammed to induce basolateral sorting, all causing intracellular misrouting (review in [121]).

One-sixteenth of all tetramers formed are wt-AQP2-only tetramers explaining the relatively milder phenotype in autosomal dominant NDI compared to autosomal recessive NDI [24, 52, 75].

Recently, Calvanese et al. using an in silico automated method for modeling point mutations in protein structures, classified *AQP2* mutations into four main groups according to their molecular effect: mutations affecting (1) the pore, (2) tetramer assembly, (3) monomer folding, and (4) signal loss for protein phosphorylation [16]. The majority of the *AQP2* mutations they modelled are known to cause autosomal recessive NDI (12/14), while two mutations cause autosomal dominant NDI. Their findings were in line with the results of in vitro functional studies mentioned above. Remarkably, they also revealed a clear correlation between the type of mutation-induced structural defect and the experimentally observed phenotypes; mutations affecting the monomer folding cause the most severe phenotypes. These mutants are nonfunctional due to their misfolding and retention in the ER. The functionality of the mutants affecting the NPA motifs and the ar/R selectivity filter is also seriously impeded. However, mutations affecting other features of the pore, such as its dimension, or mutations affecting the tetramer assembly are associated with milder phenotypes, with resulting mutant AQP2 proteins partially retaining their water channel functionality.

Differential Diagnosis between the X-Linked and the Autosomal Forms of NDI

With a few exceptions, no differences in clinical symptoms between X-linked and autosomal recessive forms of NDI can be observed nor in the time of onset of the disease. Only in a minority of patients with the X-linked form of NDI, namely, those individuals carrying *AVPR2* mutations with partial insensitivity to AVP (Table 2), the disease onset is not directly after birth but later in childhood. In general, the initial symptoms in most autosomal dominant cases also appear later in childhood.

Male patients with X-linked NDI can be discriminated from patients with autosomal recessive NDI on the basis of their extrarenal reaction to the intravenous administration of the synthetic AVPR2-vasopressin analogue 1-deamino-8-D-arginine vasopressin (DDAVP). Patients with autosomal recessive NDI show a decrease in blood pressure, accelerated heart rate, and increases in von Willebrand factor, factor VIII, and tissue-type plasminogen activator levels, whereas in all studies of patients with X-linked NDI, these extrarenal responses are absent as a result of an extrarenal mutant AVPR2 [67]. In female patients, the interpretation of this intravenous DDAVP test is more complicated. Although absence of the extrarenal responses to intravenous administration of DDAVP in females clearly points to the presence of an AVPR2 defect, a normal response cannot be interpreted as indicative of a defect beyond AVPR2 and thus an AQP2 defect. The discrepancy between the renal and extrarenal response to DDAVP in these female *AVPR2* mutation carriers might be explained by variability in the pattern of X-inactivation between different tissues.

Nephrogenic Diabetes Insipidus in Females

Several families have been described in which females show classical clinical and laboratory features of NDI. After the identification of *AQP2* mutations as a cause for autosomal recessive NDI and in some cases for autosomal dominant NDI, a satisfying explanation for the complete manifestation of the disease in some females had been found. However, several families have been reported in which symptomatic females do not have an AQP2 defect but are heterozygous for an AVPR2 defect [27, 97]. In some of these women, maximal urinary osmolality after DDAVP administration does not exceed 200 mosmol/L. Of interest, in some of the reported families, asymptomatic female family members shared the same *AVPR2* mutation with the manifesting females. The most likely explanation for the existence of different phenotypes in carriers of an *AVPR2* mutation, varying from no symptoms to complete manifestation of the disorder, is skewed X-inactivation [77]. This hypothesis was underlined by studies investigating the X-inactivation patterns in peripheral blood leukocytes of female carriers via the detection of a methylated trinucleotide repeat in the human androgen receptor gene [106]. In asymptomatic females, random X-inactivation was found, while in most female carriers who showed clinical NDI symptoms, skewed X-inactivation patterns occurring preferentially to normal X alleles were recognized. In a few females with overt clinical NDI, however, random X-inactivation was identified [82].

In conclusion, clinical NDI phenotypes may correlate with the X-inactivation patterns in females with heterozygote *AVPR2* mutations. In some female carriers, however, the clinical phenotype cannot be predicted by evaluation of X-inactivation patterns in peripheral blood cells, probably due to the fact that X-inactivation ratios within an individual may vary between different tissues.

Nephrogenic Diabetes Insipidus: Treatment

Conventional Treatment

Symptomatic treatment of NDI is aimed to achieve normovolemia by replacing urinary water losses and reducing urinary volume. Adequate supply of fluid to prevent dehydration is the most important component of the therapy. For reducing urine output, a low-solute diet is applied to diminish the renal osmole load and decrease obligatory water excretion. Initially, a diet low in sodium (1 mmol/kg per day) as well as protein (2 g/kg per day) was recommended. However, severe limitations of dietary protein may introduce serious nutritional deficiencies. Therefore, it is preferable to prescribe dietary restriction of sodium only.

Diuretics such as hydrochlorothiazide (2–4 mg/kg per 24 hours) were the first class of drugs shown to be effective in lowering the urine volume in NDI. When combined with a reduction of salt intake, hydrochlorothiazide reduces urine volume by 20%–50% of baseline values. However, thiazide-induced hypokalemia may cause further impairment or urine concentrating ability in patients with NDI. Another possible risk associated with hypokalemia is cardiac arrhythmia. Simultaneous administration of potassium salt is therefore advised in patients with hypokalemia. Very low daily sodium intake in combination with thiazide diuretics should be avoided to prevent the development of hyponatremia.

There is ample evidence that the combined administration of hydrochlorothiazide with either a prostaglandin-synthesis inhibitor such as indomethacin (2 mg/kg per 24 hours) or the potassium-sparing diuretic amiloride is much more effective in reducing urine volume than the thiazide diuretics alone [44, 60]. Prolonged use of prostaglandin-synthesis inhibitors, however, might be complicated by gastrointestinal and hematopoietic side effects. Gastrointestinal complaints and complications include anorexia, nausea, vomiting, abdominal pain, ulceration, perforation, and hemorrhage. Hematopoietic reactions include neutropenia, thrombocytopenia, and, rarely, aplastic anemia. In addition, renal dysfunction has been described during indomethacin therapy, most often consisting of a slight reduction in GFR.

In patients who are not tolerating indomethacin, selective inhibitors of cyclooxygenase-2 (COX-2) might be helpful [91]. Caution in using indomethacin and selective COX-2 inhibitors in NDI is warranted as their administration can potentially lead to the acute deterioration of renal function in dehydrated patients.

Amiloride counterbalances the potassium loss from prolonged use of thiazides and thus prevents hypokalemia. Since amiloride appears to have only minor long-term side effects, the combination of hydrochlorothiazide (2–4 mg/kg/24 hours) with amiloride (0.3 mg/kg/24 hours) is the first choice of treatment. Our personal experience of more than 20 years with the amiloride-hydrochlorothiazide combination, however, indicates that amiloride is less well tolerated in young children below the age of 4–6 years because of persistent nausea. Therefore, we advise the temporary use of the combination of indomethacin-hydrochlorothiazide in these young children.

For a long time, the following mechanism for the antidiuretic effect of thiazides in NDI has been proposed: thiazides reduce sodium reabsorption in the distal tubule by inhibition of the NaCl cotransporter (NCC). This subsequently results in increased sodium excretion, extracellular volume contraction, decreased glomerular filtration rate, and increased proximal sodium and water reabsorption. Consequently, less water and sodium reach the collecting tubules, and less water is excreted. This long-standing hypothesis has been challenged by Magaldi, who reported new insights into the possible mechanism of action, based on microperfusion studies in rat inner medullary collecting duct (IMCD) [69]. In these studies, it was shown that in the absence of vasopressin, hydrochlorothiazide, when added to the luminal side, increased osmotic and diffusional water permeabilities, thus decreasing water excretion. When prostaglandins were added, the effect of thiazides decreased. This finding may offer one explanation why indomethacin potentiates the effect of thiazides in NDI [69]. Antidiuretic effect of thiazides is associated with an increase in AQP2 expression in collecting duct cells [54]. Long-term side effects of chronic thiazide administration such as hyperuricemia, alterations in serum lipid spectrum, and glucose intolerance should be monitored.

Although the drugs mentioned above reduce urine excretion, they are unable to achieve urine volumes produced in healthy individuals. Therefore, many young patients with NDI require nasogastric tube or gastrostomy to provide adequate nutrition and fluid supply [20]. Consequently, current research focuses on methods to treat NDI on a more causative level than solely try to fight the symptoms.

Therapeutic Strategies for Treatment of X-Linked NDI

Because in vitro expression studies reveal that the majority of *AVPR2* mutations in X-linked NDI result in normal protein that is retained within the endoplasmic reticulum (ER), agents that restore plasma routing are under investigation as potential treatments. Promising agents are cell-permeable AVPR2 antagonists and agonists that in vitro rescue the intracellular retention of several AVPR2 mutants [6, 45, 101]. An important problem with the antagonists is that once the mutant AVPR2 is rescued to the basolateral membrane, the antagonist needs to be displaced by high concentrations of AVP/DDAVP to induce cAMP signaling. Therefore, low-affinity antagonists are believed to have the highest clinical value. However, their efficiency in rescuing is lower than that of high-affinity ligands, and the high concentrations required to be administered for sufficient activity by low-affinity antagonists might lead to severe complications in patients. The use of non-peptide agonists has somewhat circumvented this problem since they do not need displacement to activate AVPR2. All high-affinity agonists have been shown to induce receptor maturation as well as translocation to the plasma membrane and to elicit a cAMP response [45].

The feasibility of treatment with these so-called pharmacological chaperones has been tested in vivo. In individuals with NDI who have missense *AVPR2* mutations, Bernier et al. showed that treatment with a non-peptide V_{1a} receptor antagonist had beneficial effects on urine volume and osmolality starting a few hours after administration. However, the long-term effect of this drug could not be tested because the clinical development of this V_{1a} receptor antagonist was interrupted during the course of this study as a result of possible interference with the cytochrome P450 metabolic pathway [6]. Tolvaptan, an AVPR2 antagonist used in hyponatremia and polycystic kidney disease, was recently found in in vitro transfected cells, to rescue the function of the M272R mutation, by allowing both proper glycosylation maturation and membrane sorting and response to DDAVP, but has not been tested yet in NDI patients bearing this mutation [95].

Remarkably, certain non-peptide AVPR2 agonists such as OPC51, VA88, and VA89 were shown to be able to intracellularly stimulate AVPR2 and increase cAMP production and AQP2 translocation to the apical membrane [100]. In contrast to pharmacochaperone-assisted folding and rescue of the receptors, the localization and maturation state of AVPR2 did not change upon activation, indicating that these compounds do not act as molecular chaperones. The mode of action by which receptors trapped intracellularly can still activate their coupled G-protein, and how this stimulates adenylate cyclase is not yet understood. Future in vivo and clinical testing has to confirm whether the pharmacological chaperones or the intracellularly acting non-peptide agonists have the desired positive effects in patients and meet the safety requirements.

In patients with X-linked NDI, bypassing AVPR2 could be an alternative way to treat the disease.

By stimulation of the E-prostanoid receptor EP4, NDI symptoms were greatly reduced in a conditional *avpr2*-deletion mouse model [64]. This was due to raised AQP2 levels, most probably as a consequence of cAMP production caused by EP4 stimulation. A similar effect was seen after stimulation of the EP2 receptor by the agonist butaprost [88]. The EP2 receptor is a more interesting candidate for treatment of NDI than the EP4 receptor since EP2 agonists have already been tested in clinical studies for other diseases and have shown promising results concerning safety issues. However, clinical trials in NDI are necessary to evaluate the effects and safety of EP2 agonists for this disorder.

Another potential therapeutic strategy bypassing AVPR2 could be an activation of the cGMP-signaling pathway. Several groups have shown that nitric oxide donors and atrial natriuretic factor stimulate the insertion of AQP2 in renal epithelial cells in vitro and in vivo via a cGMP-dependent pathway without increasing the expression of *AQP2* [13] and the selective cGMP phosphodiesterase inhibitor sildenafil citrate (Viagra) prevents degradation of cGMP resulting in increased membrane expression in AQP2 in vitro and in vivo [15]. In a small number of NDI patients subjected to clinical trials with sildenafil citrate, no decreases in urine volume or increases in urine osmolality were observed (personal communication in [80]). Alternative AVP-independent strategies are the use of calcitonin, which has a vasopressin-like effect on AQP2 trafficking and urine concentrating ability via cAMP-mediated mechanism [14] and of various statins (simvastatin, fluvastatin) that were reported to increase AQP2 expression and water reabsorption in the kidney via an as yet unknown mechanism [65, 94]. Using a systemic high-throughput chemical screening procedure, Nomura et al. identified AG-490 (an epidermal growth factor receptor (EGFR) inhibitor) as a compound that stimulates AQP2 exocytosis, induces AQP2 membrane accumulation, and stimulates urine concentration in an AVP-independent manner [86]. The EGFR inhibitor erlotinib has been shown to increase AQP2-mediated renal water reabsorption, reduce urine volume, and increase urine osmolality in mice with lithium-induced NDI [17]. The most recent approach to potentially treat X-linked NDI is based on the activation of adenosine monophosphate kinase, an energy-sensing kinase that phosphorylates the Na-K-2CL cotransporter NKCC2 in the thick ascending limb of Henle's loop, by the oral antidiabetic drug metformin. Klein et al. showed that metformin increased osmotic water permeability by increasing AQP2 phosphorylation and accumulation in the apical membrane [56]. Efe et al. demonstrated that metformin improved urine concentrating ability in *avpr2* KO mice [25]. Despite these promising results in in vitro studies and in animal models, none of these compounds have yet been translated into therapy of NDI.

Therapeutic Strategies for Treatment of Autosomal NDI

Similar to AVPR2 mutants, the majority of AQP2 mutants causing autosomal recessive NDI are missense mutations that lead to aberrant folding of AQP2 in the ER. Hence, finding substances that are able to re-establish natural AQP2 folding holds comparable promises for treatment of recessive NDI as it has been shown for the X-linked form. In CHO and MDCK cells, glycerol has proven the applicability of chemical chaperones to AQP2 by restoring ER export in high concentrations [117].

Yang et al. described partial restoration of cellular AQP2 processing upon treatment of conditional AQP2-T126M knock-in mice with an Hsp90 inhibitor, 17-allylamino-demethoxygeldanamycin (17-AAG), eventually resulting in improved urinary concentrating ability [124]. The precise explanation underlying the beneficial effect of this Hsp inhibitor remains to be elucidated. Furthermore, it is not unlikely that Hsp90 inhibition may have severe side effects that outweigh the advantages [115]. Therefore, lengthened studies addressing safety issues of Hsp90 or other chaperone inhibitors have to be conducted in order to elucidate the applicability of these compounds in NDI therapy.

Based on the improvement of AVP-dependent cAMP signaling of collecting duct cells in a hypercalcemia-induced NDI mouse model, Sohara et al. also tested the phosphodiesterase-4 inhibitor rolipram in the knock-in dominant NDI mice [112]. Their data indicated that rolipram is able to increase cAMP levels leading to increased AQP2 phosphorylation and translocation to the apical membrane. Phosphodiesterase-4 is a common protein that also is involved in immunosuppressive and anti-inflammatory pathways, and therefore, its inhibition may have severe side effects. Rolipram has been tested in two male patients with X-linked NDI and did not cause any relief of symptoms [9], but the potential for other PDE inhibitors in the treatment of NDI needs to be examined further. The polyuria described in the patient bearing the autosomal dominant AQP2-E258K mutation [81] is now well controlled by DDAVP, 20 micrograms intranasally bid (D.G. Bichet, personal information).

Therapeutic Strategies for NSIAD

Fluid restriction is the mainstay of treatment for NSIAD [93]. It allows controlling hyponatremia and reducing the risks of neurological complications. This may be challenging, particularly in infants in which excessive fluid restriction may limit caloric intake. Oral urea in these patients may improve urine output by inducing osmotic diuresis [28, 41].

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Pediatric Tubulointerstitial Nephritis

45

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Abstract

Tubulointerstitial nephritis (TIN) is an immunologically mediated inflammation of renal tubulointerstitium with relative sparing of glomeruli and vessels. Clinical symptoms and laboratory findings of TIN are often unspecific, resulting in a delayed diagnosis, which may lead to a poorer clinical outcome. Renal biopsy is the gold standard of the diagnosis and shows an inflammatory interstitial infiltrate and interstitial edema. Drug-induced TIN accounts for most cases, with antibiotics, proton-pump inhibitors, and NSAIDs as leading etiologic agents. TIN can be diagnosed in association with infections, systemic disease, uveitis, and toxic factors. TIN can be acute and reversible if the offending factor is withdrawn, or may represent a chronic process leading to irreversible renal failure. The role of corticosteroids and other immunosuppressive agents in the treatment of acute TIN is controversial.

Keywords

Tubulointerstitial nephritis · Interstitial inflammation · Uveitis · Drug induced disease · TINU syndrome · Tubulointerstitial fibrosis · Kidney biopsy · Sjogren syndrome · Toxic nephropathy · DRESS syndrome · Urine beta 2 microglobulin

Introduction

Tubulointerstitial nephritis (TIN) is an immunologically mediated inflammation of the renal interstitium that can be the cause of acute kidney injury. Tubules and vessels are involved in the inflammatory process to a variable extent [1, 2]. Since around 85% of kidney tissue is made up of tubules and the surrounding interstitium, these structures are of crucial importance in renal disease processes [3]. Clinical symptoms and laboratory findings of tubulointerstitial nephritis are often unspecific, so that this can result in a delayed diagnosis and a poorer clinical outcome [4]. Renal biopsy is the gold standard of the diagnosis of tubulointerstitial nephritis and shows an inflammatory interstitial infiltrate and interstitial edema [5]. In rare cases, the interstitial inflammation leads to chronic renal impairment [6]. TIN is characterized histologically by inflammation and damage of tubulointerstitial structures, with relative sparing of glomerular and vascular elements. It can be acute or chronic in its appearance and course. The likelihood of chronicity increases with systemic inflammatory or rheumatologic diseases and delayed removal of the causative medication in drug-induced TIN. Biopsy proven drug-induced acute TIN accounts for most cases (71%), of which antibiotics represent the most common responsible class of drug followed by proton-pump inhibitors and NSAIDs [7]. In adult patients with AKI, AIN represents 18% to 27% of biopsied cases [8, 9, 10, 11].

In recent years, the prevalence of TIN seems to continue to increase, especially in patients over 65 years of age, most likely due to the widespread use of antibiotics, nonsteroidal anti-inflammatory drugs (NSAID), and proton pump inhibitors (PPIs), which are common triggers of TIN [6, 12]. With the exception of TINU syndrome (tubulointerstitial nephritis with uveitis, see below), the disease is significantly more common in adults than in children.

This chapter focuses on acute TIN.

Structure and Function of the Tubulointerstitial Compartment

The tubulointerstitial compartment consists of tubular epithelium, vascular structures, lymphatics, and interstitium, together accounting for more than 90% of the kidney volume [13]. The interstitium fills the space between tubular and vascular basement membranes. It is composed of extracellular matrix with matrix proteins, including collagen I, III, V, and VII, fibronectin, osteopontin, and proteoglycans, which are expressed normally in very small amounts, interstitial fluid, and a large number of intrinsic and infiltrating cells. It provides structural support for the nephrons and capillaries and plays an important role in the transport of solutes and production of cytokines and hormones such as prostaglandins and erythropoietin [11]. Separation into cortical and medullary interstitia allows countercurrent medullary solute trapping and the development of the corticomedullary osmotic gradient, establishing kidney concentrating ability [14].

Multiple cell types are important components of the interstitium. Perivascular cells (pericytes) reside between macula densa and afferent arterioles, participating in renal autoregulation. Fibroblasts constitute the major cell type in the interstitium. They determine matrix production and degradation and form a continuous network throughout the kidney [15]. Erythropoietin production occurs in the peritubular interstitial fibroblasts [16] stimulated by hypoxia through the activation of hypoxia-inducible transcription factors (HIF) [17].

Circulating monocytes migrate into the kidney and transform into renal interstitial macrophages, which can differentiate into inflammatory M1 or reparative M2 phenotypes [18]. Dendritic cells are bone-marrow derived antigen-presenting cells that express MHC class II [19]. They interact with T cells within the interstitial lymphatic network to process antigens and elicit inflammatory responses [20]. Following tubular damage and inflammatory signals interstitial fibroblasts proliferate, change to the myofibroblast phenotype, and increase their collagen production, potentially resulting in renal fibrosis [16]. Tubular epithelial cells have antigen-presenting capability, can secrete various growth factors and cytokines, interact with interstitium, and participate in interstitial inflammation in various pathologic conditions [21].

According to these multiple functions and properties of tubulointerstitial compartment, its damage leads to different metabolic disturbances, including impaired renal function and concentrating ability, anemia, and possible fibrosis development and progression to chronic kidney disease (CKD).

Pathophysiology of TIN

Pathogenesis

Tubulointerstitial nephritis is characterized by an inflammatory cell infiltrate, interstitial edema, and tubulitis. This process is often immune mediated, driven by activation of both innate and adaptive immune, or nonimmune responses. If prolonged, TIN leads to interstitial fibrosis as the final common pathway. The previously proposed leading mechanism by allergic hypersensitivity with systemic and tissue eosinophilia in drug-induced TIN is not universal, and different pathways in drug-induced conditions can be seen, as with nonsteroidal anti-inflammatory drugs (NSAIDs), where interstitial eosinophils infiltration lacks, implicating a non–type IV drug-hypersensitivity reaction [22]. However, the possible role of mast cell activation by IgE has been shown in a human study [23].

The composition of the interstitial infiltrate is similar, whether the initiating cause of injury is toxic, ischemic [24], autoimmune [25], or secondary to protein overload [26]. It is possible that immune responses to injured interstitial cell neoantigens are a final common pathway for interstitial injury.

Recent experimental studies have uncovered a new pathogenetic mechanism called "necroinflammation," defined as immune response to necrosis in living organism, which links primary tubular damage with an interstitial inflammatory response [27]. This mechanism was recently reviewed in the context of drug-induced interstitial nephritis [28]. "Necroinflammation" is triggered by a specific form of necrotic cell death (necroptosis) characterized by plasma membrane rupture and proinflammatory cytokine release, increased vascular permeability, and recruitment of innate immune effector cells. Initial renal damage leads to inflammation and cause additional tubular cell necroptosis, setting an injury amplification loop. If not arrested, it will eventually lead to organ dysfunction. Necrotic cell particles can form danger-associated molecular patterns (DAMPs) and activate specific pattern recognition receptors (PRR), including several Toll-like cellular membrane receptors and intracellular receptors. These receptors are expressed by immune or nonimmune cells, including many native kidney cells. This ligand-receptor interaction triggers the release of various pro-inflammatory mediators and cytokines leading to an inflammatory response [29].

Studies from experimental models of tubulointerstitial disease indicate that both cell-mediated and humoral immune mechanisms are relevant pathways for inducing renal injury [30].

Cell-mediated immune response to initial injury promotes interstitial inflammation and tubular injury via infiltration of lymphocytes, neutrophils, macrophages, mast cells, and eosinophils. Of these, T cells including $CD4^+$ and $CD8^+$ constitute 80% of the infiltrating cells [31]. Cell-mediated responses are initiated by T-cell recognition of antigens presented on antigen-presenting dendritic cells and renal tubular epithelial cells that express MHC molecules, as previously mentioned. Costimulatory molecules CD80 and CD86 are needed for this process [32]. Under physiologic conditions antigen-presenting ability of renal cells is limited, preventing inflammatory response. Genetic susceptibility may play a factor in antigen expression and consequent immune response [33], as well as drugs, infectious agents, or molecular mimicry that may serve as inciting antigens of cell-mediated responses targeting the kidney [34]. Monocytes and macrophages infiltrating the renal interstitium cause cellular damage by generating reactive oxygen species, nitric oxide, complement factors, and proinflammatory cytokines [35]. These cells can also affect supporting matrix and vasculature through the expression of metalloproteinases and vasoactive peptides. Proper macrophages function is important for the clearance of danger-associated molecular patterns (DAMPs), pathogen-associated molecular patterns, and neutrophils from injured tissue preventing persistent inflammation [36]. Interstitial macrophages have phenotypic and functional heterogeneity and may also have a beneficial antifibrotic role in inflammation [37].

Tubulointerstitial inflammation may develop also in the absence of antigenic stimulation [38]. There is growing evidence for the role of innate immune sensors (TLR2, TLR4, and MyD88 signaling) and the inflammasome complex in the initiation of tubulointerstitial injury [39]. In nonimmune-mediated TIN, ammonia can activate the alternative complement pathway [40], which indicates the importance of correction of metabolic acidosis in the prevention of progressive damage to the kidneys by diminishing ammonia production.

Antibody-mediated TIN is rarely seen. Immune deposits on renal biopsy can be seen along the tubular basement membrane (TBM) and represent specific anti-TBM antibodies or reflect immune complexes deposited in this area. Immune complexes in interstitial nephritis are probably formed in situ, similar to what has been shown in post-streptococcal glomerulonephritis [41]. The deposits generally consist of IgG and complement factor C3. However, TIN with IgM deposits has been described [42]. Tubulointerstitial antigen (TINag) on proximal tubule's BM is the major target of anti-TBM antibody-mediated TIN in humans [43]. Anti-TBM antibodies can be seen in association with drug-induced TIN and more commonly in the setting of renal transplantation due to the presence of foreign antigens in the transplanted kidney [44].

Proteinuria-Induced Tubular Cell Injury

The potential inflammatory and fibrogenic role of abnormal glomerular filtrate should also be considered. In glomerular injury the extrusion of plasma contents directly into the adjacent tubulointerstitium results in inflammation and scarring [45]. Proteinuria is an important factor in progression of tubulointerstitial disease. Mechanisms underlying nephrotoxic injury induced by excessive proteinuria involve numerous pathways of cellular damage. Urinary proteins activate proximal tubular cells into acquiring a pro-inflammatory phenotype [46]. Several in vitro studies have demonstrated that certain urinary proteins (particularly albumin) can stimulate proximal tubular cells to synthesize chemokines, such as monocyte chemoattractant protein-1 (MCP-1), RANTES, and others that recruit monocytes and T cells and interleukin-8 that attract neutrophils [47, 48]. Autophagy is one of the upregulated mechanisms of protein-induced renal injury as well [49]. Dose- and duration-dependent induction of apoptosis was also demonstrated in cultured proximal tubular cells after protein overload [50].

Tubulointerstitial Fibrosis Development

In the majority of patients, tubulointerstitial inflammation is controlled by the immune system and does not lead to progressive damage. However, if inflammation fails to terminate and self-limiting healing does not occur, a maladaptive process typically leads to abnormal healing, chronic inflammation, and fibrosis. Progressive tubulointerstitial fibrosis is the final common pathway for all kidney diseases leading to CKD [51]. Fibrogenesis initiates in small areas of inflammation and then expands to become diffuse if profibrotic drivers persist [52]. The process of tubulointerstitial fibrosis involves the loss of renal tubules and the accumulation of fibroblasts and matrix proteins, such as collagen (types I–V and VII), fibronectin, and laminin [53].

Fibroblasts in tubulointerstitium may arise from transformation of tubular epithelium in the process of epithelial–mesenchymal transformation (EMT) [54], activated by immune-mediated mechanisms and various cytokines secreted by interstitial cells. A number of proteins, including Wnt proteins, integrin-linked kinases, IGF-1 and IGF-2, epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), and TGF-β, are among the modulators of EMT [55]. One of the strongest morphogenic cytokines driving tubulointerstitial fibrosis is angiotensin II [56]. Vascular pericytes may also differentiate into fibroblasts [57]. Transformed fibroblasts migrate to interstitium influenced by chemoattractants and begin to deposit fibronectin matrix and collagen. Interstitial M1 inflammatory and reparative profibrotic M2 macrophages interact with fibroblasts via various cytokines, determining the extent of renal fibrogenesis.

In parallel to fibrosis formation, the effect of cytokines on tubular cells can result in their atrophy and destruction. Thus, tubular atrophy and interstitial fibrosis coexist. Tubular damage plays a central role in the progression of CKD. The central role in this process is thought to belong to proximal tubule as was recently reviewed [42]. Activation and dedifferentiation of proximal tubular cells mediate multiple components of the fibrogenic response. Remnant nephron hypertrophy, alteration in tubuloglomerular feedback, impaired autoregulation of renal blood flow, and disconnection of proximal tubuli from glomeruli (“atubular glomeruli”) are well-recognized interrelated consequences of tubulointerstitial fibrosis that lead to CKD progression.

Chronic Hypoxia in Fibrosis

Hypoxia contributes directly to the progression of tubulointerstitial fibrosis by simulating EMT and promoting matrix accumulation from new fibroblasts [58]. Interstitial fibrosis reduces peritubular vascular supply leading to starvation of tubules, tubular atrophy, and loss. Hypermetabolism of remaining tubular cells requires increased oxygen consumption further promoting hypoxia [59].

Hypoxia exacerbates ER stress, leading to the autophagy of proteins and mitochondria and release of reactive oxygen species (ROS) [42]. One of ROS actions is the stabilization and generation of hypoxia inducible factor (HIF), which promotes extracellular matrix expression [60], by profibrotic factors expression stimulation [61]. Cyanotic heart disease can be a clinical example of chronic hypoxia. Nephropathy has long been recognized as a potential complication of long standing cyanotic congenital heart disease [62]. Animal models of hypoxia-induced changes in congenital heart disease demonstrate that renal tubular structure is abnormal and interstitial fibrosis is present after a relatively short period of tissue hypoxia (28 days in rats) [63].

Epidemiology

There are no reliable data on the incidence and prevalence of TIN in either adults or children. For adults, the proportion of TIN in all biopsies of 0.5–2.6% can be estimated from the evaluation of biopsy registers, while it is responsible for 5–19% of acute kidney injury [6]. The collection of epidemiological data is made more difficult by the fact that not all patients are biopsied, the clinical symptoms are often unspecific, and there are clear regional differences with regard to the etiology of the disease [9].

No prospective studies are available in pediatric patients. A single large center trial from North America reports on 133 biopsy-proven acute TIN cases over a period of 18 years. A pediatric single center experience over 3 years more than three decades ago, found acute TIN in seven patients, which comprised 7% of all biopsies performed over that period.[9] A recent contemporary series form a referral center in the UK reports on similar numbers (10 cases over an 8 year period) [64].

Pathology

The "gold standard" of acute TIN diagnosis is renal biopsy (Fig. 1). The characteristic finding of acute TIN is interstitial infiltration by inflammatory cells. These cells can cross tubular basement membrane (TBM) and enter tubules ("tubulitis"), which is often accompanied by degenerative tubular changes. Interstitial edema may be present, and the degree of fibrosis depends on the pathologic process persistence. The interstitial infiltrate is composed of T lymphocytes (mostly CD4^{+} and CD8^{+}), and less amount of macrophages, plasma cells, neutrophils, and eosinophils, depending on TIN etiology. Eosinophils can present in tubulointerstitial infiltrate in cases of drug-induced TIN, tubulointerstitial nephritis and uveitis (TINU), and IgG4-related kidney disease, but their absence does not exclude these diagnoses (like in the case of NSAIDs and PPI-induced TIN) [65]. Neutrophils are suggestive of an infectious etiology. Non-caseating granulomas can be occasionally found in drug-induced and sarcoidosis-related TIN [66]. Medications more commonly associated with granuloma formation include anticonvulsants, antibiotics, NSAIDs, allopurinol, immune checkpoint inhibitors, and diuretics. Glomeruli and blood vessels are typically spared. However, in NSAID-induced acute TIN, glomerular lesions such as minimal change disease or membranous nephropathy may be present, correlating with significant proteinuria.

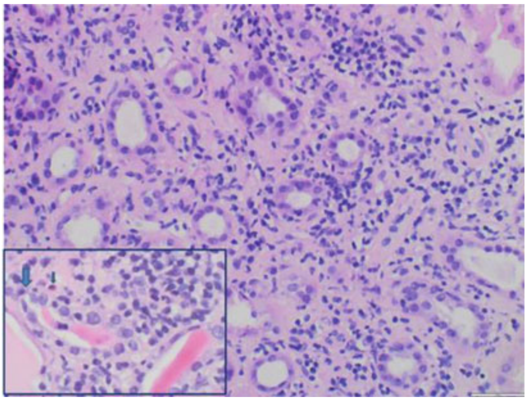

Fig. 1 Histopathological picture of acute TIN: white blood cells infiltrate the tubulointerstitial space, with increased inter tubular space (magnification: X100). Left lower quadrant: enlargement (X400): small arrow: eosinophil; large arrow: plasma cell

Immunofluorescence is typically unremarkable. Occasionally positive staining for IgG and

C3 is present on TBMs in the cases of anti-TBM antibodies mediated disease. Significant IgG4 positivity in plasma cells in interstitial infiltrate is observed with IgG4-related kidney disease [67].

Etiology

The causes of tubulointerstitial nephritis vary. Associated, possible, and systemic diseases are known to cause interstitial nephritis. Furthermore, TIN occurs in connection with a mostly anterior uveitis in the context of the TINU syndrome (see below). If the cause of TIN cannot be revealed, it is referred to as an idiopathic form.

Drug-Induced TIN

See Table 1 for an ever-growing list of drugs associated with TIN. The most common medications associated with TIN are antibiotics, NSAID, and proton pump inhibitors [68]. A retrospective analysis of more than 1000 pediatric AKI cases revealed that exposure to NSAID (usually in the proper therapeutic dosage) was responsible for AKI in and 6.6% of cases, when excluding complex patients with multifactorial AKI. Chronic use of PPI has been shown in several large population studies (involving adults) to be associated with incident CKD [69]. Using two large adult patients' databases and comparing exposure to PPIs vs H2 blockers or by propensity score matching, use of PPIs conferred an increased risk for CKD [65].

DRESS Syndrome

Drug reaction with eosinophilia and systemic symptoms (DRESS) is a rare but potentially fatal adverse drug reaction with variable renal involvement. The most common medications associated with this entity are anticonvulsants and allopurinol. DRESS diagnosis can be difficult if not suspected, since there could be a significant lag period (weeks to months) between beginning of treatment and the appearance of symptoms. TIN has also been described as the renal manifestation in DRESS [70].

Secondary Immune Acute TIN

Systemic Lupus Erythematosus (SLE)

SLE is the most important cause of acute TIN seen in association with glomerulonephritis in children. In few cases a predominant acute TIN has been described in SLE patients [71]. Tubulointerstitial immune deposits may be present in up to 60% of SLE patients who are biopsied and correlate with both severity of interstitial inflammation and degree of functional renal impairment. As is the case with other glomerular diseases, severe tubulointerstitial involvement with fibrosis is a poor prognostic indicator for renal function [72].

Sjogren Syndrome

Primary SS (pSS) is a chronic autoimmune disorder characterized by lymphocytic infiltration of exocrine glands, resulting in significant loss of secretory function, usually the salivary and lacrimal glands, resulting in oral and eye dryness. The course of the disease is also characterized by the occurrence of extraglandular features, with frequent multi-organ involvement, including the kidney. However, overt renal disease is a rare event during pSS [73]. In a nationwide survey from France of patients with pSS and with biopsy-proven renal involvement ($n = 95$), the lowest quartile of patients was less than 18 years old. In this cohort, 75% of kidney biopsies showed TIN [74]. In many cases renal tubular acidosis is seen [75].

TINU

Young patients with uveitis should be screened for renal tubulointerstitial co-involvement, using urine low molecular weight proteinuria and serum creatinine testing [76]. Vice versa, all patients with TIN should be screened by an ophthalmologist for uveitis. The co-appearance of inflammatory conditions in two remote organs speaks in favor of an autoimmune tendency. Indeed, correlation between IL10 gene (responsible for acute inflammatory response

Table 1 List of medications associated with acute TIN. * Pediatric cases reported

Anti-inflammatory drugs and analgesics
- Benoxaprofen
- Diclofenac
- Diflunisal
- Fenoprofen
- Floctafenine
- Glafenine*
- Ibuprofen*
- Indometacin
- Ketoprofen
- Mefenamic acid
- Naproxen*
- Niflumic acid*
- Phenazone
- Phenylbutazone
- Piroxicam
- Rofecoxib
- Sulfinpyrazone
- Sulindac
- Suprofen
- Tolmetin*
- Zomepirac

B-lactam antibiotics
- *Cephalosporins*
  - Cefaclor
  - Cefotaxime
  - Cefoxitine
  - Ceftriaxone
  - Cephalexin
  - Cephaloridine
  - Cephalothin
  - Cephradine
- *Penicillins and derivatives*
  - Penicillin G*
  - Amoxicillin
  - Ampicillin*
  - Carbenicillin
  - Cloxacillin*
  - Flucloxacillin
  - Methicillin*
  - Mezlocillin
  - Nafcillin*
  - Piperacillin-tazobactam
  - Oxacillin

Other antibiotics
- Azythromycin
- Aztreonam
- Chloramphenicol
- Ciprofloxacin
- Clarithromycin
- Co-trimoxazole
- Erythromycin*
- Ethambutol
- Gentamycin
- Isoniazid
- Lincomycin
- Loracarbef*
- Nitrofurantoin
- Norfloxacin
- Piromidic acid
- Polymyxin sulfate
- Rifampin
- Spiramycin
- Sulfonamides*
- Sulfadiazine
- Trimethoprim-sulfamethoxazole*
- Vancomycin
- Trimethoprim-sulfamethoxazole*
- Vancomycin

Antivirals
- Acyclovir
- Indinavir

Tetracyclines
- Minocycline*

Anticonvulsants
- Carbamazepine*
- Lamotrigine
- Phenobarbital*
- Phenytoin*
- Sodium valproate*

Proton pump inhibitors
- Omeprazole
- Lansoprazole
- Esomeprazole
- Pantoprazole

Diuretics
- Chlorthalidone
- Ethacrynic acid
- Furosemide
- Thiazides
- Tienilic acid
- Triamterene

Antineoplastic agents
- Adriamycin
- Bevacizumab
- Carboplatin
- Cytosine arabinoside
- Ifosfamide
- Immune-check point inhibitors
- Interferon
- Methotrexate
- Tyrosine kinase inhibitors

5-aminosalicylates
- Mesalazine*
- Sulfasalazine

Other drugs
- Aldomet
- Allopurinol
- Amlodipine
- Amphethamine
- Anti-CD4 antibodies
- Aspirin
- Azathioprine
- Captopril
- Chlorprothixene
- Cimetidine
- Clofibrate
- Clozapine
- Coumadin
- Crack cocaine
- Creatine
- Cyclosporine*
- Diazepam
- Doxepin
- Etanercept
- Haloperidol
- Haloperidol
- Heroin
- Herbal medicines (aristolochic acid)
- Imipramine
- Pamidronate
- Phenazone
- Phenindione*
- Phenylpropanolamine*
- Propranolol
- Propylthiouracil
- Protease inhibitors
- Quinine
- Ranitidine
- Streptokinase

downregulation) polymorphisms and TINU was found in a Finnish cohort [77]. Uveitis and TIN may not appear at the same time. Another study from Finland reported in 26 children with an original diagnosis of acute TIN that uveitis may develop late and without recurrence of renal dysfunction [78]. In a retrospective patient series from France, which included adults and adolescents, oral corticosteroid therapy was associated with less relapses of uveitis, but did not affect renal function course [79].

Inflammatory Bowel Disease (IBD)

IBD may cause different renal manifestations, including obstructive uropathy and glomerular diseases (mainly IgA nephropathy and minimal change disease). TIN was found in 19% of kidney biopsies, in most of them in association of mesalazine use, the commonest nonsteroidal anti-inflammatory agent used in IBD [80].

Kawasaki Disease (KD)

Even though sterile pyuria is a common manifestation of KD, AKI has rarely been reported, usually in association with acute heart failure and multiple organ dysfunction. However, a minority of case reports include TIN as an etiology for renal dysfunction [81].

Allograft Nephropathy

This topic is reviewed in Chap. “Allograft Nephropathy” in this book.

IgG4-Related Kidney Disease

IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory disorder involving almost any organ in the body. A literature search identified 25 children with this disease. The most common manifestations included orbital disease (44%) and autoimmune pancreatitis (12%) [82]. TIN is the commonest form of renal involvement in IgG4-related disease. It is characterized by a dominant infiltrate of IgG4-positive plasma cells in the interstitium and storiform fibrosis. However, IgG4 positive plasma cells may also be seen in SLE, Sjogren syndrome, and ANCA-associated vasculitis [83].

Toxic Nephropathy[1]

Numerous medical and non-medical substances may be associated with AKI by different mechanisms, and its clinical picture may be similar to acute TIN. This topic is reviewed extensively in another book’s chapter, but several medications and substances will be mentioned in this chapter too.

Aristolochic Acids

The previously termed Chinese herb nephropathy and Balkan nephropathy entities are nowadays known to be associated with the ingestion of aristolochic acid, and it is now termed aristolochic acid nephropathy (AAN). AAN may cause urothelial cancer as well as CKD [84]. Its clinical presentation may be acute or chronic [85], and it has also been described in children and adolescents [86].

Old and Novel Anti-Cancer Drugs

It is important to remember that some of the medications used in cancer (including Ifosfamide, BCG, tyrosine kinase inhibitors, premetrexed, and oxaliplatin) may impair kidney function not just through a tubulo-toxic mechanism but also through TIN, some of them acute and reversible [87]. Use of immune checkpoint inhibitors (anti CTLA4 and PD1 inhibitors) may cause different renal side effects, including AIN [88]. Even though the use of these medications group has been increasing significantly in pediatrics in the last years [89], there have been no reports yet on renal side effects by these novel agents.

Infection Associated TIN[2]

This topic is also extensively reviewed in another book chapter.

Hantavirus infections are endemic worldwide and may cause AKI in association with an epidemic-infectious pattern. It is usually associated with thrombocytopenia. In Western Europe the Puumala subtype of hantavirus is common. A patient series from Germany compared the clinical symptoms and course in 295 adults and 22 children. AKI, thrombocytopenia, sudden onset of fever, and back/loin pain were similarly common in both adults and children. Whereas adults presented more frequently with arthralgia and visual disturbances, abdominal pain and nausea/vomiting could be detected more often in children. No significant differences were found in the incidence of complications (hemodialysis, long-term outcome of kidney function, length of hospital stay) [90].

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[1] There is a chapter on “Allograft Nephropathy” by Scott Sutherland

[2] See Chap. 26, “Infections That Affect the Kidney (Non-viral),” by V Koch and 27, “Viral Infections and the Kidney,” by P Ray

Leptospira

TIN is a common clinicopathological finding in leptospirosis. Its renal clinical manifestations include nonoliguric AKI, hypokalemia, sodium, and magnesium wasting. Primary injury of the proximal convoluted tubules is regarded as the hallmark of the kidney in leptospirosis [91].

Group A Streptococcus

Acute TIN secondary to streptococcal infection has been seldom reported in past years. However, TIN was first described as a clinical entity in patients with scarlet fever. Ellis et al. described 13 children with biopsy-proven TIN, seven of whom had clinical and/or laboratory signs of streptococcal infection [92]. Histopathological analysis in this entity showed no staining for either immunoglobulins or complement factors, but immunohistochemical studies may reveal the presence of streptococcal pyrogenic exotoxin B in the tubulointerstitial compartment.

TIN of Unknown Etiology

Mesoamerican Nephropathy

This disease affects mainly agricultural workers and has been originally described in central America, but was later described also in Egypt, India, and Sri Lanka. Disease etiology was originally suspected to be due repeated dehydration episodes, and most affected patients presented already at the CKD stage. In a small prospective study of patients with unexplained AKI living in central America, 11 consecutive patients underwent a kidney biopsy, which revealed primary interstitial nephritis with intact glomeruli [93], suggesting that this disease undergoes initially through an active, inflammatory phase. This emphasizes again the overlap between non-glomerular AKI, toxic nephropathy, and “allergic” TIN.

Genetic Causes of Tubulointerstitial Diseases

Several genetic factors have been associated with development of chronic forms of TIN that inevitably lead to end-stage renal disease [94, 95, 96, 97]. These disorders are reviewed in other chapters of the book [98].

Clinical Presentation

Tubulointerstitial nephritis often presents with unspecific clinical symptoms. A late diagnosis with delayed initiation of therapy and poor outcome can be the result.

Acute TIN is responsible for about 10% of AKI cases. Sometimes TIN may be the presenting symptom of a systemic disease (including TINU, but also other rheumatic diseases), which will develop within months to years from the initial diagnosis of acute TIN [99].

The clinical manifestations of TIN are variable, and differentiation between acute and chronic TIN may be difficult. Renal impairment severity may range from mild, as asymptomatic urinary abnormalities, mild azotemia and nonoliguric or oliguric AKI. These nonspecific clinical findings emphasize the need for a renal biopsy to make a definitive diagnosis in questionable cases. However, in children with mild impairment of kidney function, if improvement in kidney function is noticed after withholding the suspected offending agents or, if a clear-cut clinical picture of acute allergic TIN is seen (leading to therapy initiation), a biopsy is not needed. Symptoms may develop several days and at times weeks after exposure to the inciting agent. Systemic manifestations of a hypersensitivity reaction, such as fever, rash, and arthralgia, are variable findings. Although these symptoms are more likely to occur in drug-induced acute TIN, it is seldom (only in 10%) seen as a triad [100]. The rash may be maculopapular, morbilliform, or pruritic-urticarial, and preferentially affect the trunk and proximal extremities. Arthralgia is sometimes seen. Acute TIN associated with infection may present with extrarenal manifestations, such as fever and sore throat, fatigue, anorexia, weight loss, nausea, and vomiting. Flank pain and even hematuria may be seen in the minority of cases [101]. Hypertension and edema are seldom noted, except in specific drug-induced lesions, such as the nephrotic syndrome associated with nonsteroidal anti-inflammatory drugs (NSAIDs), or in TIN secondary to glomerular disease.

Decrease in glomerular filtration secondary to the tubular injury results in elevated serum creatinine concentration. Urinalysis may show active (leukocytes, red blood cells) or relatively bland changes. Associated non-kidney abnormalities include normochromic, normocytic anemia, which at times may be hemolytic [102]. Leukocytosis may occur, and peripheral eosinophilia is seen in a minority of patients (in some of them as part of the drug reaction, eosinophilia and systemic symptoms syndrome – DRESS). Elevated serum IgE titers have been reported in less than 50% of biopsy-proven cases and are suggestive of drug-induced TIN. Anti-DNA antibodies, antinuclear antibodies, and complement levels are normal in most forms of TIN, unless associated with a systemic autoimmune disorder. Renal ultrasound examination usually demonstrates normal or enlarged kidneys and at times hyperechogenicity, depending on the extent of tubulointerstitial inflammation and edema. Gallium citrate scanning may be useful in distinguishing acute TIN from other common causes of AKI. However, increased uptake on gallium scan is a relatively nonspecific finding and may be seen in cases of allograft rejection, acute pyelonephritis, and severe minimal change nephrotic syndrome. Therefore, this modality is no longer used and many times TIN can only be made by renal biopsy.

Urinary Biomarkers

Since renal tubular epithelial cell damage by infiltrating inflammatory cells is the predominant pathophysiology, laboratory abnormalities in TIN may reflect injury to specific nephron segments involved in the inflammatory process. Proximal tubule injury results in Fanconi syndrome with impaired urinary reabsorption of glucose, bicarbonate, phosphorus, amino acids, alpha-1 and β2-microglobulin, and uric acid. Damage to the loop of Henle may result in magnesium and sodium losses. Distal tubular lesions result in renal tubular acidosis, impaired potassium excretion, sodium wasting, and a urine-concentrating defect.

Even though kidney biopsy remains the gold standard for TIN diagnosis, some classical and novel biomarkers for TIN may serve in the diagnosis, prognosis, and follow-up of this disease, as recently reviewed [103]. Sterile leukocyturia is an almost universal finding in drug-related and especially in antibiotic-related acute TIN [104]. Due to the type IV hypersensitivity basis of drug-induced acute TIN, eosinophiluria was considered a classical biomarker in acute TIN, but it is no longer considered sensitive or specific [105]. Detection of LMW proteins in the urine may be signs of ongoing renal tubulointerstitial inflammation or fibrosis, or both, which supports the use of these biomarkers in follow-up of activity of TIN [106]. Urinary β2M and serum creatinine levels were shown to be sensitive and relatively simple diagnostic screening tools for detecting TINU syndrome in young children with uveitis [76]. Monocyte chemoattractant protein-1 (MCP-1), an important chemoattractant involved in interstitial inflammation, was evaluated in several works. Higher urinary MCP-1 levels were shown in a cohort of 40 patients with drug-induced ATIN compared to controls, in correlation with the severity of the acute lesions in kidney biopsies from these patients [107]. In another cohort of 113 acute TIN patients from different causes, higher MCP-1 serum and urine levels were also detected [108]. Urinary MCP-1 concentration finely correlated with the amount of tubulointerstitial but not with the glomerular infiltrate in a cohort of patients with glomerular autoimmune diseases [109]. In humans with ureteropelvic junction obstruction, urinary MCP-1 is increased and improves after obstruction surgical alleviation [110]. Higher serum and urine levels of tumor necrosis factor alpha (TNF-α) and urinary IL-9 have been reported in patients with acute TIN [111] [112]. The value of serum TNF-α levels in the follow-up of a case of TINU dramatically decreased during the first week after treatment initiation.113 Other unspecific tubular AKI biomarkers have also been evaluated in acute TIN. Urinary NGAL, NAG, and α1-microglobulin levels were found to be significantly elevated in patients with drug-induced TIN compared to controls.106 Urinary KIM-1 is a specific biomarker for acute renal tubular injury, including in patients with TIN, whereas urinary soluble complement terminal attack complex (sC5b-9), a marker of complement activation, is valuable in reflecting severe interstitial inflammation. The combination of these two biomarkers may be used to identify patients with TIN at an acute injury stage.114

Cellular activation markers on the surface of lymphocytes after incubation with the offending drug – such as CD25, CD69, or human Leukocyte antigen (HLA)-DR – may indicate hypersensitivity.115 Activation of T cells after drug exposure triggers a specific pattern of cytokine secretion that can be detected, using enzyme- linked assays. Release of high amounts of IL-5, IL-10, and IFN-γ, in the setting of hypersensitivity has been reported.116

Diagnostic Approach

Figure 2 provides an overview on a possible diagnostic pathway, primarily based on an ultrasound examination, urinary dipstick analysis, and measurement of urinary alpha-1 or beta-2 microglobulin/ creatinine ratios in children with AKI. After excluding other causes like pre-renal failure, glomerular disease, or urosepsis, in most cases a renal biopsy is needed to diagnose TIN, unless a clearly drug-induced cause can be found, that can be treated by drug discontinuation. After diagnosis of TIN, efforts should be done to identify the cause for TIN and consequently, the treatment of TIN and/or the underlying cause/disease could be started.

Treatment

The most important aspect in the therapy of tubulointerstitial nephritis is its early diagnosis.[1] With the drug-induced forms, a complete or partial recovery of kidney function can be expected if the TIN is recognized in good time and the drug causing the problem is discontinued immediately. The likelihood of recovery in kidney function depends largely on the duration of the kidney damage prior to diagnosis and should ideally be less than 2 weeks. About a third of patients develop chronic kidney impairment.117 In the case of systemic illnesses or infections, treating the underlying illness has priority.

The role of corticosteroids in the therapy of tubulointerstitial nephritis is controversial. There are no randomized, controlled studies on the use of corticosteroids, and retrospective studies in adults have provided contradicting results. For example, a study on 60 adult patients with tubulointerstitial nephritis of different etiologies could not demonstrate any effect of corticosteroid treatment on the outcome (measured as serum creatinine 1, 6, and 12 months after the onset of the disease).118 In contrast, a multicenter retrospective study with 61 adult patients with drug-induced TIN showed a significantly higher risk of permanent impairment of kidney function and chronic dialysis in patients who were not treated with corticosteroids. There was also a significant correlation between the latency to the start of steroid treatment and long-term kidney function: an interval of more than 7 days between discontinuation of the drug and the start of steroid therapy increased the risk of incomplete recovery of kidney function.119 In a prospective pediatric study with 17 patients, corticosteroids were shown to accelerate the recovery of kidney function, especially in patients with severe renal impairment.120

There is no consensus on the duration and dosage of corticosteroid therapy, neither for adults nor for children. Common practice is oral steroid therapy in tapering doses that, depending on the course, extend over several weeks. Intravenous steroid bolus therapy can precede oral treatment if the course is severe.

Mycophenolate mofetil (MMF) is used successfully in individual cases in patients with steroid-resistant courses or contraindications for corticosteroids.121 To date, however, there is too little evidence for the widespread use of MMF.

Intravenous immune globulin (IVIG): A case series from Israel describes seven children with

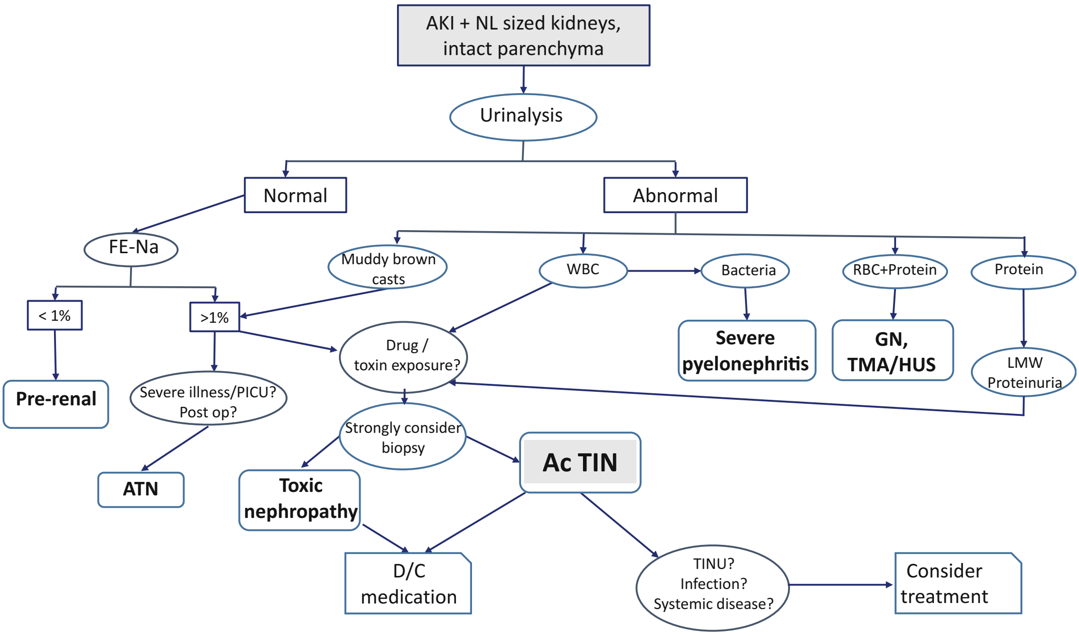
AKI + NL sized kidneys, intact parenchyma
Urinalysis
Normal
Abnormal
FE-Na
< 1%
>1%
Pre-renal
Severe illness/PICU? Post op?
ATN
Muddy brown casts
WBC
Bacteria
RBC+Protein
Protein
Severe pyelonephritis
GN, TMA/HUS
LMW Proteinuria
Drug / toxin exposure?
Strongly consider biopsy
Toxic nephropathy
Ac TIN
D/C medication
TINU? Infection? Systemic disease?
Consider treatment

Fig. 2 Approach to suspected acute TIN, algorithm. Abbreviations: *AKI*, acute kidney injury; *ATN*, acute tubular necrosis; *D/C*, discontinue; *GN*, glomerulonephritis; *TMA*, thrombotic microangiopathy; *HUS*, hemolytic uremic syndrome; *LMW Proteinuria*, low molecular weight proteinuria; *Ac TIN*, acute Tubulointerstitial Nephritis

severe DRESS in spite of offending drug withdrawal and systemic corticosteroid therapy, who benefited substantially from a 1 to 2 g/kg course of IVIG.122

Prognosis

The long-term prognosis for ATIN is generally favorable, with full kidney recovery within 1 month in most cases.123 However, some patients may develop progressive chronic kidney disease. Immunosuppressive therapy may be indicated for severe or prolonged disease.

In summary: Early diagnosis is crucial for therapy and improved prognosis of TIN. Clinical symptoms such as fever, fatigue, nausea, vomiting, and volatile rash in combination with increased kidney function parameters and characteristic urine findings (no hematuria, trace proteinuria by dipstick, but significant α1/β2 microglobulinuria) indicate tubulointerstitial nephritis. The medical history should include questions about previous medication use (especially antibiotics, NSAID), symptoms of infection, chronic diseases, and ocular symptoms. Discontinuation of the triggering substance or treatment of the underlying disease is of crucial importance in the therapy of TIN.

Oral and intravenous corticosteroids are widely used as drug therapy, although randomized controlled studies on steroid therapy of TIN do not yet exist.

Cross-References

- ▶ Autosomal Dominant Tubulointerstitial Kidney Disease
- ▶ Evaluation and Management of Acute Kidney Injury in Children
- ▶ Infections That Affect the Kidney (Nonviral)
- ▶ Lupus Nephritis
- ▶ Nephrotoxins
- ▶ Onconephrology
- ▶ Renal Transplantation: Non-infectious Complications and Long-Term Outcome
- ▶ Viral Infections and the Kidney

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Part VI

Omeostasis

Sodium and Water Disorders: Evaluation and Management

46

Michael L. Moritz

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Abstract

Dysnatremias are among the most common electrolyte abnormalities encountered in hospitalized patients. Proper fluid management and early detection are important in the prevention and treatment of dysnatremias. Virtually all acutely ill patients are at risk for developing hyponatremia due to numerous hemodynamic and non-hemodynamic stimuli for arginine vasopressin (AVP) release. One of most common causes of hyponatremia in hospitalized patients is the syndrome of inappropriate antidiuresis (SIAD). An elevated fractional excretion of urate is helpful in establishing a diagnosis of SIAD. A major contributing factor to the development of hospital-acquired hyponatremia is the administration of hypotonic fluids. Recent clinical practice guidelines have recommended isotonic maintenance fluids for the prevention of

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hospital-acquired hyponatremia. Hyponatremic encephalopathy is the most serious complication of hyponatremia. Consensus guidelines have recommended intermittent bolus therapy with 3% sodium chloride for the treatment of hyponatremic encephalopathy. Excessive therapy of severe and chronic hyponatremia can also lead to neurologic injury due to cerebral demyelination. Hospital-acquired hypernatremia occurs in patients who have restricted access to fluids in combination with ongoing free water losses. A group at high-risk for developing hypernatremia in the outpatient setting is that of the breastfed infant due to insufficient lactation. Patients at risk for severe hospital-acquired hyponatremia are those with diabetes insipidus. A useful test for distinguishing central from nephrogenic diabetes insipidus is plasma copeptin testing. Copeptin can be used as a surrogate marker of AVP as it is easier and more reliable to measure.

Keywords

Hypernatremia · Hyponatremia · SIAD · Diabetes insipidus · Fluid therapy

Water Homeostasis

The plasma osmolality is maintained within a narrow range, 275–290 mOsm/kg. The hypothalamus senses alterations in osmolality, which leads to changes in thirst and release of arginine vasopressin (AVP) in order to return the osmolality to normal. Osmoreceptors are located in the anteroventral third ventricle of the hypothalamus and AVP is synthesized in the vasopressinergic neurons of supraoptic and paraventricular nuclei of the hypothalamus [1]. AVP is stored and subsequently released from the posterior pituitary (Fig. 1). The gene that encodes AVP is located on chromosome 20. The biosynthesis of AVP proceeds by way of a polypeptide precursor. This prohormone contains the AVP moiety, a protein known as neurophysin II (NPII) and a glycoprotein. This prohormone ultimately folds and dimerizes and is cleaved into AVP, where it is stored in the posterior pituitary until it is release into the cavernous sinus and superior vena cava [2].

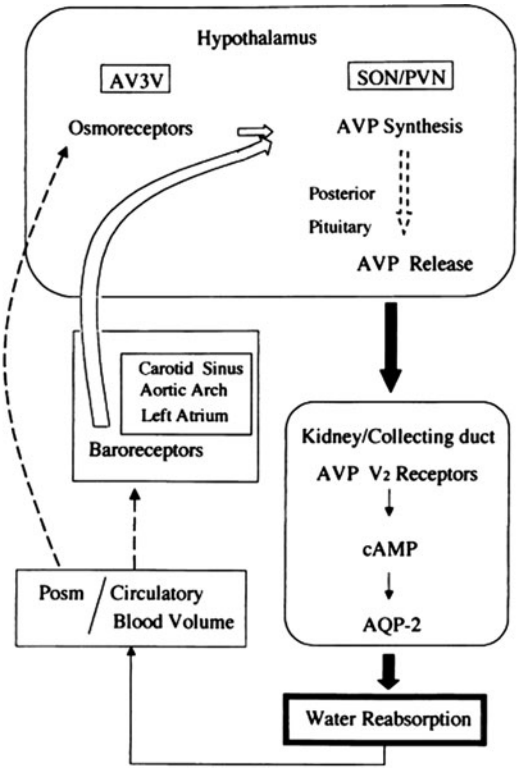

Fig. 1 AVP and water homeostasis: Anteroventral third ventricle (AV3V), supraoptic and paraventricular nuclei (SON/PVN). (From: Ishikawa SE, Schrier RW. Pathophysiological roles of arginine vasopressin and aquaporin-2 in impaired water excretion. Clin Endocrinol (Oxf) 2003;58(1):1–17) [1]

AVP is the primary determinant of free water excretion. Its major renal affect is to increase water permeability in the medullary and collecting tubules via the insertion of the aquaporin 2 (AQP2) water channel on the apical surface of the cortical collecting duct (Fig. 1) [3]. The action of AVP is mediated via the vasopressin V2 receptor that is located on the basolateral surface of the principal cells. The V2 receptor is coupled to a guanine-nucleotide-binding protein, Gs. Binding of AVP to the V2 receptors results in activation of adenylate cyclase with increase in cAMP and activation of c-AMP-dependent protein kinase A (PKA). Activated PKA phosphorylates the serine residue at the C-terminus of the AQP2 protein, which leads to insertion of AQP2 on apical surface of the principal cells of the cortical collecting duct resulting in increased water permeability. Water movement across the basolateral membrane is facilitated by AQP3 and AQP4. Any defect along this pathway will prevent AQP2 expression and will result in a renal concentrating defect. On the other hand, process that increase the expression of AQP2 result in impaired ability to excrete free water.

Hyponatremia

Hyponatremia, serum sodium <135 mEq/L, is one of the most common electrolyte disorders in both ambulatory and hospitalized patients. The incidence of hyponatremia is approximately 2% in the general population, increasing with age [4], and 15% in unselected hospitalized children [5]. The majority of hyponatremia is hospital-acquired- and related to hypotonic fluid administration, occurring in approximately 30% of children receiving intravenous fluids [6]. Hyponatremia is an independent prognostic factor for risk-adjusted mortality in ambulatory and hospital settings, and particularly in patients with end-stage liver disease, congestive heart failure, pneumonia, and advanced kidney disease [7]. Mild and chronic hyponatremia can result in subtle neurologic impairment and can lead to increased bone fragility resulting from bone demineralization in both children and adults [8, 9]. Hyponatremic encephalopathy is the most serious complication [10].

Pathogenesis

The body's primary defense against hyponatremia is the kidney's ability to generate dilute urine and excrete free water. Rarely is excess ingestion of free water alone the cause of hyponatremia, as an adult with normal renal function can typically excrete over 15 L of free water per day. It is also rare to develop hyponatremia from excess urinary sodium losses in the absence of free water ingestion. In order for hyponatremia to develop it requires relative excess of free water intake in conjunction with an underlying condition that impairs urinary free water excretion (Table 1). Renal water handling is primarily under the control of AVP that is produced in the hypothalamus and released from the posterior pituitary [11]. AVP release impairs water diuresis by increasing the permeability to water in the collecting tubule. There are numerous hemodynamic and non-hemodynamic stimuli for AVP release, and this places almost all acutely ill patients at risk for hyponatremia [12]. The body will attempt to preserve the extracellular volume at the expense of the serum sodium, and therefore a hemodynamic stimulus for AVP production will override any inhibitory hypo-osmolar effect of hyponatremia [13]. There are numerous stimuli for AVP production that make many hospitalized children at risk for hyponatremia due to the syndrome of inappropriate antidiuresis (SIAD).

Diagnosis

Before embarking on a therapeutic regimen, it is vital to confirm that hyponatremia is associated with hypoosmolality. Hyponatremia can be associated with either normal or elevated serum

Table 1 Disorders in impaired renal water excretion

1. Effective circulating volume depletion
(a) Gastrointestinal losses: vomiting, diarrhea
(b) Skin losses: cystic fibrosis
(c) Renal losses: salt-wasting nephropathy, diuretics, cerebral salt wasting, hypoaldosteronism
(d) Edematous states: heart failure, cirrhosis, nephrosis, hypoalbuminemia
(e) Decreased peripheral vascular resistance: sepsis, hypothyroidism
2. Thiazide diuretics
3. Renal failure
(a) Acute
(b) Chronic
4. Non-hypovolemic states of ADH excess
(a) Syndrome of Inappropriate of Antidiuresis (SIAD)
(b) Nausea, emesis, pain, stress
(c) Postoperative state
(d) Cortisol deficiency
5. Nephrogenic Syndrome of Inappropriate Antidiuresis (NSIAD)

osmolality (Fig. 2). Common reasons for the latter are hyperglycemia, severe hyperproteinemia, or hyperlipidemia. Hyperglycemia results in hyperosmolality with translocation of fluid from the intracellular to the extracellular space, resulting in a 1.6 mEq/L fall in the serum sodium for every 100 mg/dL elevation in blood glucose concentration above normal [14]. Severe hyperlipidemia, hypercholesterolemia, hyperproteinemia, or radiocontrast and IV immunoglobulin infusions can displace plasma water, resulting in a decreased sodium concentration (pseudohyponatremia) with a normal serum osmolality [15]. Serum sodium is currently measured by either direct or indirect-reading ion-selective electrode potentiometry [16]. The direct method will not result in a diagnosis of pseudohyponatremia, as it measures the activity of sodium in the aqueous phase of serum only. Whole blood sodium, blood gas, and point of care analyzers employ the direct methods and are not subject to pseudohyponatremia. Most laboratories use the indirect method, which can result in pseudohyponatremia as the specimen requires a predilution. If hyponatremia is associated with hypoosmolality (true hyponatremia), the next step is to measure the urinary osmolality to determine if there is an impaired ability to excrete free water (osmolality >100 mOsm/kg).

The information that is most useful in arriving at a correct diagnosis of hyponatremia is a detailed history of fluid balance, weight changes, medications (especially diuretics), and underlying medical illnesses. Hyponatremia is usually a multifactorial disorder and a detailed history helps identify sources of salt and water losses, free water ingestion, and underlying illnesses that cause a non-osmotic stimulus for vasopressin production. An assessment of the volume status on physical examination and measuring the urinary electrolytes and osmolality is helpful. In patients in whom hyponatremia is due to salt losses (e.g., diuretics), signs of volume depletion may be absent on examination, as the volume deficit may be nearly corrected due to oral intake of hypotonic fluids if the thirst mechanism is intact, or in patients receiving hypotonic IV fluids.

In general, urinary sodium concentration less than 30 mEq/L is consistent with effective circulating volume depletion, while urine sodium greater than 30 mEq/L is consistent with renal tubular dysfunction, use of diuretics, or SIAD [17]. Numerous factors can affect the urine sodium concentration, making interpretation difficult. Therefore, the timing of the urinary measurements in relation to therapy with diuretics, IV fluid boluses, or fluid and sodium restriction is important. In some cases, estimation of intravascular volume status by measurement of the central venous pressure or sonography of the inferior vena cava may be helpful.

SIAD

SIAD is one of the most common causes of hyponatremia in hospitalized children [18]. The causes are numerous, and virtually every hospitalized patient should be considered at risk of SIAD. SIAD is most often the result of central nervous system disorders, pulmonary disorders, malignancies, and medications. Many common disorders are associated with SIAD, such as pneumonia, bronchiolitis, asthma, positive pressure ventilation, CNS infections, and head trauma. Elevated blood levels of AVP and hyponatremia are associated with febrile illnesses, inflammation, and infections. Numerous medications are associated, including anticonvulsants, antidepressants, antipsychotics, opioids, chemotherapeutic agents, proton pump inhibitors, nonsteroidal anti-inflammatory agents, and recreational drugs. Chief medications associated with SIAD in children are carbemazepine, oxcarbazepine, vincristine, and cyclophosphamide. Common physiological stimuli for AVP release that can lead to an SIAD-like state are nausea, vomiting, pain, stress, hypoxia, and hypoglycemia [19]. Postoperative hyponatremia is frequently associated with an SIAD-like state due to numerous stimuli for AVP production including pain, stress, nausea, vomiting, opioids, and positive pressure ventilation [20, 21]. Exercise-associated hyponatremia is reported in children and collegiate athletes [22–24].

The hallmarks of SIAD are: mild volume expansion with low to normal plasma concentrations of creatinine, urea, uric acid, and potassium;

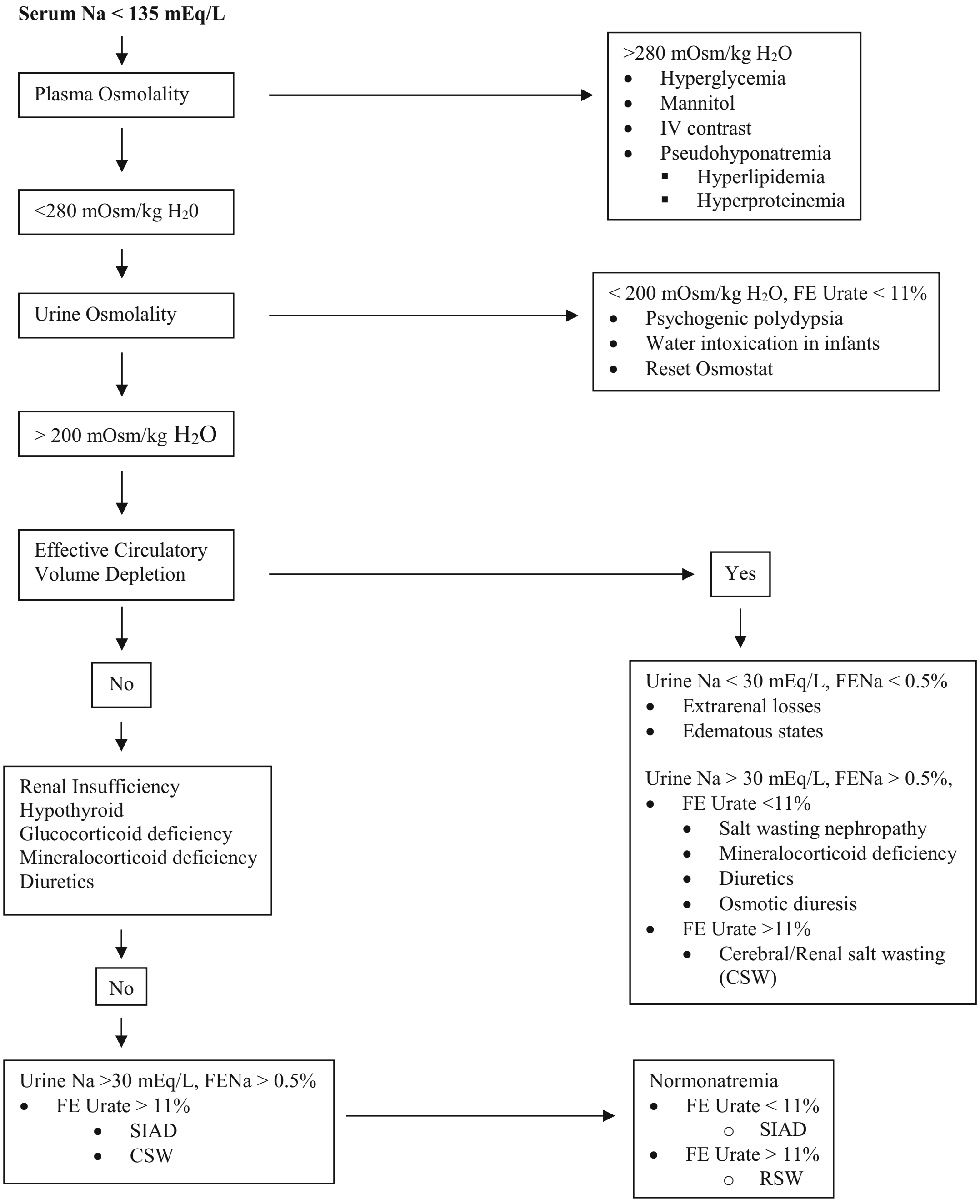
Serum Na < 135 mEq/L
Plasma Osmolality
>280 mOsm/kg H2O
Hyperglycemia
Mannitol
IV contrast
Pseudohyponatremia
Hyperlipidemia
Hyperproteinemia
<280 mOsm/kg H20
Urine Osmolality
< 200 mOsm/kg H2O, FE Urate < 11%
Psychogenic polydypsia
Water intoxication in infants
Reset Osmostat
> 200 mOsm/kg H2O
Effective Circulatory Volume Depletion
Yes
Urine Na < 30 mEq/L, FENa < 0.5%
Extrarenal losses
Edematous states
Urine Na > 30 mEq/L, FENa > 0.5%,
FE Urate <11%
Salt wasting nephropathy
Mineralocorticoid deficiency
Diuretics
Osmotic diuresis
FE Urate >11%
Cerebral/Renal salt wasting (CSW)
No
Renal Insufficiency
Hypothyroid
Glucocorticoid deficiency
Mineralocorticoid deficiency
Diuretics
No
Urine Na >30 mEq/L, FENa > 0.5%
FE Urate > 11%
SIAD
CSW
Normonatremia
FE Urate < 11%
SIAD
FE Urate > 11%
RSW

Fig. 2 Evaluation of hyponatremia

impaired free water excretion with normal sodium excretion which reflects sodium intake [25]; and hyponatremia that is relatively unresponsive to sodium administration in the absence of fluid restriction.

There are no urinary or plasma biomarkers that have a sufficiently high sensitivity and specificity to diagnose SIAD, independent of clinical assessment. Measurements of AVP or copeptin, a surrogate marker of vasopressin, have not proved useful for the diagnosis [26, 27]. The biochemical parameters suggestive of SIAD in adults include: spot urine sodium concentration >30 mEq/L, fractional excretion of sodium >0.5%, fractional excretion of urea >55%, fractional excretion of urate (FEUrate) >11%, and plasma uric acid <4 mg/dl (Fig. 2) [17]. The specificity and sensitivity of these biomarkers have not been evaluated in children. The urinary biomarker most specific for diagnosing SIAD is an elevated FEUrate [27, 28]. FEUrate is unlikely to be elevated with diuretic use or adrenal insufficiency, whereas urinary sodium biochemistries may be high. Adult parameters for FEUrate cannot be used in infants and children with renal disease, as they have higher levels [29].

Cerebral Salt Wasting Syndrome (CSW)

Another condition that is indistinguishable from SIAD is CSW. The latter is less common, and primarily but not exclusively associated with central nervous system disease; the term renal salt wasting is more appropriate [30]. The condition is chiefly seen in neurosurgical patients, in particular adults with subarachnoid hemorrhage. CSW is best described as a syndrome of inappropriate natriuresis [31]. Inappropriate and excessive release of natriuretic peptides leads to natriuresis and volume depletion. This stimulates a secondary neurohormonal response, with increase in the renin angiotensin system and in AVP production. Signs of volume depletion are not always present, and there are no investigations that consistently distinguish CSW from SIAD at the time of presentation [30]. Natriuretic peptides are known to inhibit the renin angiotensin system and the secretion and action of AVP, and to increase GFR [32]. This complex mechanism can lead to biochemical features that are indistinguishable from those of SIAD, with normal blood urea and low uric acid, as well as normal plasma renin, aldosterone, and AVP in the setting of volume depletion. Patients with CSW have higher urine volume and sodium excretion than SIAD [33]. The severity of CSW is variable; some patients may not show hyponatremia [34].

Hospital-Acquired Hyponatremia

Hospital-acquired hyponatremia is of concern in acutely ill children as the standard of care in pediatrics had been to administer hypotonic maintenance fluids containing 0.2–0.45% sodium chloride [12, 35, 36]. The safety of this approach had never been established. Hospitalized children have numerous nonosmotic stimuli for increased production of AVP that place them at risk for hyponatremia. Critically ill children are at particularly higher risk as most have multiple nonosmotic stimuli for AVP secretion (e.g., pulmonary disorders, mechanical ventilation, intracranial injury). Hyponatremia is especially dangerous in children with underlying CNS injury such as encephalitis, where even mild hyponatremia (sodium ~130 mEq/L) may result in cerebral edema and even herniation [10]. Numerous studies have demonstrated that hypotonic fluids are associated with a high incidence of hospital-acquired hyponatremia, in both surgical and nonsurgical patients and in the intensive care unit and general pediatric ward [6, 37–39]. In 2018, the American Academy of Pediatrics issued a Clinical Practice Guideline on maintenance intravenous fluids stating that the most important measure to prevent hyponatremia is to avoid using hypotonic fluids in patients with clear risks for nonosmotic AVP secretion, and to administer isotonic saline (0.9% sodium chloride, Plasma-Lyte) instead [40]. These recommendations were based on over 20 prospective trials in ~3000 patients showing that isotonic fluids decrease the incidence of hyponatremia three to sixfold in comparison with hypotonic fluids. This has led to a practice shift with increased use of isotonic maintenance fluid in children [41].

Clinical indications to not use 0.9% sodium are primarily in disease states associated with increased free water losses, such as renal concentrating defects, voluminous diarrhea, or severe burns. Furthermore, children with neurosurgical disorders, significant congenital or acquired heart disease, hepatic or renal dysfunction, fluid overload or edema, children undergoing chemotherapy, or <28-days old may require a different maintenance fluid composition and infusion rate.

Consequences of Hyponatremia and Hyponatremic Encephalopathy

There is evidence that even mild and seemingly asymptomatic hyponatremia has deleterious consequences [7]. Hyponatremia is an independent risk factor for mortality in adults with SIAD [42]. Mild and chronic hyponatremia can results in subtle neurologic impairment leading to falls and associated bone fractures in the elderly, with increased bone fragility resulting from bone demineralization [8, 43–46]. Hyponatremia represents a significant health care burden and results in increased medical costs and hospital stays [47]. Hyponatremia might also alter the immune response, and explain the increased rate of infections in hyponatremic patients [48, 49].

The most serious complication is hyponatremic encephalopathy, which is a medical emergency that can be fatal or can lead to irreversible brain injury if inadequately treated [50]. It is important to identify patients at risk for hyponatremic encephalopathy, since this might be complicated by transtentorial herniation with death or permanent neurologic sequelae [20]. Hypoosmolality results in an influx of water into the intracellular space down a concentration gradient resulting in parenchymal cell swelling, or cytotoxic cerebral edema (Fig. 3). The brain's primary mechanism for adapting to hyponatremia is extrusion of intracellular electrolytes and osmolytes [51]. Adaptation of the brain to hyponatremia takes time; based on animal studies; it takes approximately 48 h for the brain to adapt to hyponatremia [52]. Acute hyponatremia is defined as hyponatremia of less than 48-h duration, and chronic >48-h. The brain is also vulnerable to neurologic injury from overcorrection following chronic hyponatremia [53].

While abrupt and severe hyponatremia is likely to produce brain edema, then mild and chronic hyponatremia, there are risk factors for hyponatremic encephalopathy developing at mildly hyponatremic levels or with chronic hyponatremia. Risk factors include age <16 years [54], postmenarche [20], hypoxemia [55], or underlying central nervous system disease [56]. These risk factors can either impair brain cell volume regulation or are associated with decreased intracranial capacity for brain expansion. Children are at particularly high risk for hyponatremic encephalopathy as they have a relatively larger brain-to-intracranial volume compared to adults [52, 54]. The average serum sodium in children with hyponatremic encephalopathy is 120 mEq/L [57, 58], significantly higher than in adults of ~111 mEq/L [20, 59]. More than 50% children with serum sodium <125 mEq/L develop hyponatremic encephalopathy [10]. The majority of children reported with encephalopathy were otherwise healthy. They had received hypotonic fluids in the setting of SIAD; multiple deaths were reported [10, 56, 60].

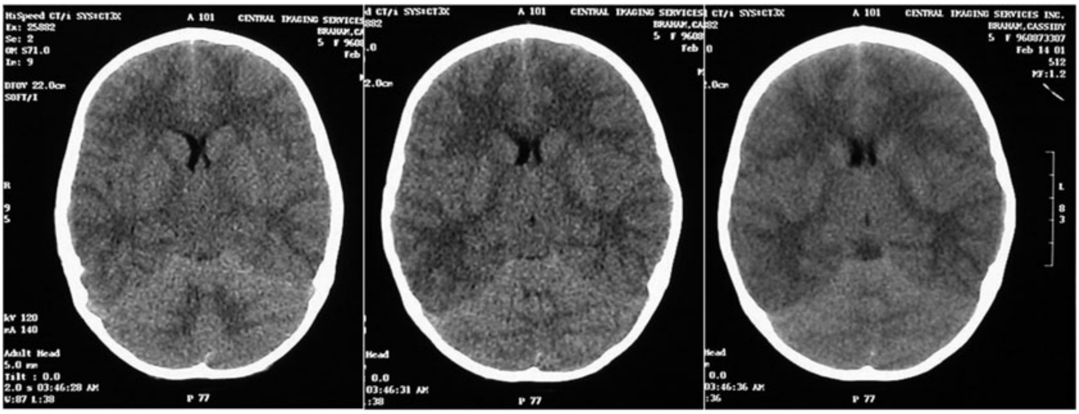

Fig. 3 Head CT demonstrating diffuse cerebral edema in a 5-year-old female who developed hyponatremic encephalopathy following a tympanoplasty. She was administered hypotonic maintenance fluids postoperatively and developed a serum sodium of 123 mEq/L

The primary symptoms of hyponatremia are those of cerebral edema, including headache, nausea, vomiting, and lethargy. Advanced symptoms of encephalopathy include seizures, coma, non-cardiogenic pulmonary edema, and decorticate posturing [56]. Cranial CT might not show mild cerebral edema, which is better detected by diffusion weighted imaging MRI [56, 61].

Treatment of Hyponatremic Encephalopathy

Hyponatremic encephalopathy is a medical emergency that requires immediate therapy with intravenous 3% sodium chloride (3% NaCl, 513 mEq/L) [62–64]. Fluid restriction, isotonic saline and vaptans have no role as initial therapy for hyponatremic encephalopathy as they are either ineffective, will not consistently increase the sodium by a sufficient degree, or do not have a rapid enough onset of action [65]. The consensus treatment for hyponatremic encephalopathy is to give repeated boluses of 3% NaCl (2 ml/kg, maximum 150 ml) in order to acutely raise the serum sodium by approximately 5 mEq/L (Table 2) [64]. This approach is indicated for both mild and severe symptoms and for acute and chronic hyponatremia. Hypertonic (3%) saline has proven to be safe and effective in treating hyponatremic encephalopathy in both children and adults [57, 66, 67]. The optimal magnitude of correction of hyponatremia is unknown. A correction of 10–20 mEq/L in the first 48 h is associated with best outcomes [59, 68, 69]. Treatment of hyponatremic encephalopathy should precede any neuroimaging studies to confirm cerebral edema and should occur in a monitored setting where the airway can be secured and serum sodium level measured frequently. Hypertonic saline can be given safely through a peripheral line in children and adults; a central line is not required for its administration.

Table 2 Treatment of symptomatic hyponatremia

1. 2 ml/kg bolus of 3% NaCl over 10 min. Maximum 150 ml
2. Repeat bolus 1–2 times as needed until symptoms improve. Goal: 5–6 mEql/L increase in serum sodium (SNa) in first 1–2 h
3. Recheck SNa following second or third bolus or Q 2 h
4. Hyponatremic encephalopathy is unlikely if no clinical improvement following an acute rise in serum sodium of 5–6 mEq/L
5. Stop further therapy with 3% NaCl boluses when patient is either:
(a) Symptom free: awake, alert, responding to commands, resolution of headache, and nausea
(b) Acute rise in sodium of 10 mEq/L in first 5 h
6. Correction in first 48 h should:
(a) Not exceed 15–20 mEq/L
(b) Avoid normo- or hypernatremia

Cerebral Demyelination Complicating the Correction of Hyponatremia

Cerebral demyelination is a rare complication following the correction of hyponatremia. Animal data has shown that correction of hyponatremia by greater than 20–25 mEq/L over 24 h can result in cerebral demyelination [68]. The condition is primarily seen in chronically hyponatremic patients of greater than 48-h duration with serum sodium <115 mEq/L. Other risk factors that increase the likelihood of the complication, independent of the degree of hyponatremia or magnitude of correction, are hypokalemia, thiazide diuretic use, severe liver disease, malnutrition, hypophosphatemia, and hypoxia [10]. Cerebral demyelination is not seen in patients with acute hyponatremia that is frequently encountered in children. In patients with severe and chronic hyponatremia, sodium should not be corrected by greater than 20 mEq/L in the first 48-h of therapy. Patients with hyponatremia due to water intoxication, diarrheal dehydration, or thiazide diuretics are at high risk for overcorrection of hyponatremia as they experience brisk free-water diuresis following volume expansion.

When symptomatic cerebral demyelination follows the correction of hyponatremia it typically follows a biphasic pattern. There is initial clinical improvement of the hyponatremic encephalopathy associated with correction of the serum sodium, which is followed by neurological deterioration 2–7 days later. Cerebral demyelination can be both pontine and extrapontine. Classic features of pontine demyelination include mutism, dysarthria, spastic quadriplegia, pseudobulbar palsy, a pseudocoma with a "locked-in stare," and ataxia. The clinical features of extrapontine lesions are more varied, including behavior changes and movement disorders. Radiographic features of cerebral demyelination typically lag behind the clinical symptoms. Cerebral demyelination is best diagnosed by MRI approximately 14 days following hyponatremia correction. The findings are symmetrical hypointense lesions on T1-weighted images and hyperintense lesions on T2-weighted images. Most patients demonstrate improvement in neurological symptoms and regression of radiographic findings.

Therapies for Hyponatremia

Hyponatremia is a multifactorial condition and its management depends on the underlying etiology. Fluid restriction is the cornerstone in the management of hyponatremia, as hyponatremia is unlikely to occur in the absence of fluid intake. Fluid restriction is indicated as part of the management of euvolemic and hypervolemic hyponatremia, as is seen in SIAD and congestive heart failure [70]. Fluid intake from all sources should be less than the daily urine output, and generally less than 600–1000 ml/day in an adult. However, this is a slow means of correcting hyponatremia and frequently ineffective.

Isotonic saline is primarily indicated for the management of hypovolemic hyponatremia as can occur with gastrointestinal losses, diuretics, or mineralocorticoid deficiency. Administering isotonic saline is useful in distinguishing hypovolemic hyponatremia from SIAD. Isotonic saline is generally ineffective in increasing the serum sodium in SIAD and can potentially worsen the hyponatremia [71]. An appreciable response in serum sodium in response to saline is consistent with volume depletion. Saline should be avoided in patients with hypervolemic hyponatremia as it can worsen fluid overload.

Studies are beginning to support the use or oral urea as practical therapy to correct asymptomatic chronic hyponatremia from SIAD [72]. Urea primarily works as an osmotic diuretic by increasing the renal solute load. A commercially available lemon-flavored urea powder drink (Ure-Na by Nephcentric LLC) is now available in the United States. In the United States it is medical food, Generally Regarded as Safe (GRAS) by the FDA, which can be purchased over the counter and used under medical supervision. It comes in 15 g single serve packets to be mixed in 3–4 ounces of water or juice. In can be used in conjunction with other therapies such as sodium chloride tablets. It is well tolerated and allows for a more liberal fluid restriction. Hypertonic saline is primarily indicated for the management of symptomatic hyponatremia, though in select cases it could be used to correct asymptomatic hyponatremia unresponsive to conventional therapies in patients with SIAD, cerebral salt wasting, and with loop diuretics in congestive heart failure.

Vasopressin-2 antagonists (vaptans) represent a relatively new class of medication for the management of SIAD. These agents antagonize the antidiuretic effect of AVP and results in a urinary free water diuresis (aquaresis) without increasing loss of electrolytes. Vaptans produce an aquaresis within 1–2 h of administration which abates within 12–24 h. When used to treat hyponatremia, vaptans result in an approximately 5–7 mEq/L increase in serum sodium within the first 24 h of administration, but the effect is variable [73]. The side effects of vaptans are increased thirst, polyuria, and dry mouth. Two vaptans are approved by the US FDA: tolvaptan for oral and conivaptan for intravenous use. Safety concerns with vaptans include elevation of alanine aminotransferase elevation and severe hepatotoxicity with long-term use, and serious overcorrection of hyponatremia has been reported. Overcorrection is of particular concern in neurologically impaired or critically ill children with restricted access to water. Vaptans are inhibitors of cytochrome P450 and should not be used in conjunction with other drugs known to be metabolized by this pathway. At present, vaptans cannot be recommended as a first-line agent in the management of SIADH, as they are expensive, not always necessary, and present safety concerns. Vaptans do appear to be a suitable second-line agent for short-term use in patients with SIAD after conservative measures have failed.

Hypernatremia

Hypernatremia is defined as serum sodium >145 mEq/L. The condition occurs in children and adults, often in the presence of restricted access to water for a variety of reasons. In most instances these patients are either debilitated by an acute or chronic illness or neurologic impairment or are at the extremes of age. Diarrheal dehydration is an important cause of hypernatremia, though is less common than previously reported, presumably due to the advent of low solute infant formulas, the increased use and availability of oral rehydration solutions, and better access to care. A group at high risk for developing hypernatremia in the outpatient setting are breastfed infants [74]. Hypernatremic dehydration results from a combination of insufficient lactation and high breast milk sodium concentration [75]. Contributing factors in the intensive care setting are excess sodium administration, renal concentrating defects, gastrointestinal fluid losses, increased insensible water losses, restricted access to oral fluids, and dialysis-related complications. Excess intake can occur via infusion of hypertonic solutions, blood products, and sodium bicarbonate. Increased insensible water losses occur with fever, tachypnea, and burns. Hypernatremia can result from intentional salt poisoning, in particular in children with gastrostomy tubes [76]. Serum sodium levels >145 mEq/L should be considered abnormal and evaluated in order to prevent the development of significant hypernatremia.

Pathogenesis

The body has two defenses to protect against developing hypernatremia: the ability to produce concentrated urine by reabsorbing filtered water and a powerful thirst mechanism. Antidiuretic hormone (ADH) release occurs when the plasma osmolality exceeds 275–280 mOsm/kg and results in a maximally concentrated urine when the plasma osmolality exceeds 290–295 mOsm/kg. Thirst is the second line of defense but provides the ultimate protection against hypernatremia. If this mechanism is intact and there is unrestricted access to free water, it is rare to develop sustained hypernatremia from either excess sodium ingestion or a renal concentrating defect.

Diagnosis

The cause of hypernatremia is usually multifactorial and a systematic approach is required to determine the underlying etiology (Fig. 4). Serum sodium, glucose, and osmolality must be simultaneously evaluated. Elevated serum sodium is always associated with hyperosmolality and should be considered abnormal. In association with significant hyperglycemia, the serum sodium concentration is depressed due to the associated translocation of fluids from the intracellular to extracellular space, and therefore true hypernatremia will be masked. Once the diagnosis of hypernatremia is established, a detailed history and review of fluid intake is obtained to determine if the patient has an intact thirst mechanism, had restricted access to fluids, or was not provided adequate free water in intravenous fluids.

The following should be evaluated: gastrointestinal losses, urinary output, dermal losses from fever or burns, diet history (including tube feedings), medication history (including diuretics), and sources of exogenous sodium. Urine volume should be measured and compared to fluid intake. Urine osmolality and electrolytes should be determined to assess if the renal concentrating ability is appropriate and to quantify urinary free water losses. Salt poisoning should be considered in cases of severe and unexplained hypernatremia. A gastric or stool sodium concentration higher than plasma sodium is virtually diagnostic of salt poisoning. Pseudohypernatremia is reported to occur with vecuronium and esmolol, due to interference with ion-selective electrodes [77].

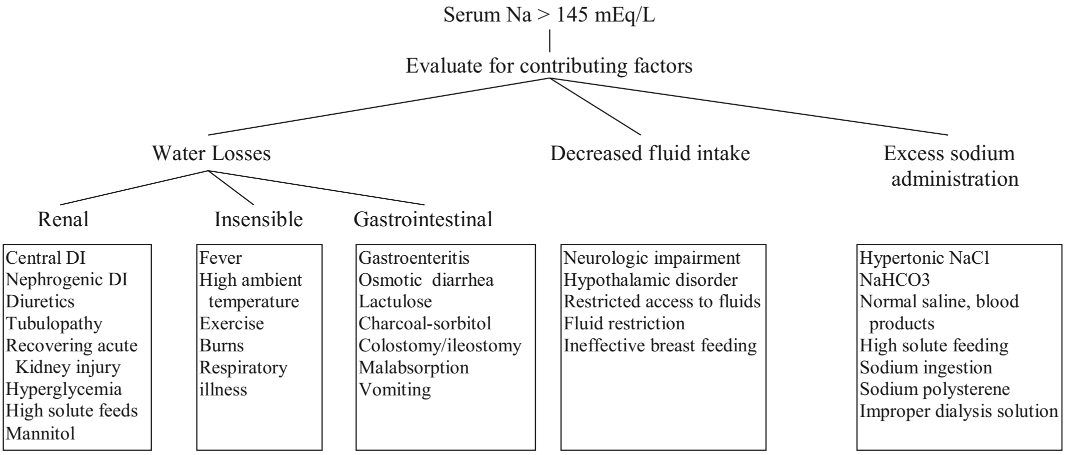
Serum Na > 145 mEq/L
Evaluate for contributing factors
Water Losses
Decreased fluid intake
Excess sodium administration
Renal
Insensible
Gastrointestinal
Central DI
Nephrogenic DI
Diuretics
Tubulopathy
Recovering acute Kidney injury
Hyperglycemia
High solute feeds
Mannitol
Fever
High ambient temperature
Exercise
Burns
Respiratory illness
Gastroenteritis
Osmotic diarrhea
Lactulose
Charcoal-sorbitol
Colostomy/ileostomy
Malabsorption
Vomiting
Neurologic impairment
Hypothalamic disorder
Restricted access to fluids
Fluid restriction
Ineffective breast feeding
Hypertonic NaCl
NaHCO3
Normal saline, blood products
High solute feeding
Sodium ingestion
Sodium polystyrene
Improper dialysis solution

Fig. 4 Diagnostic approach to hypernatremia

Diabetes Insipidus

Diabetes insipidus (DI) occurs due to either inadequate vasopressin production, termed central diabetes insipidus, or renal resistance to vasopressin, termed nephrogenic diabetes insipidus (NDI). NDI can be congenital or acquired, secondary to either: (i) resistance of AVP in the collecting tubule; (ii) impaired medullary countercurrent mechanism; and (iii) increased solute excretion by the kidney [78]. Acquired forms of NDI are commonly encountered in practice, but the congenital forms are the most severe. Hypernatremia is a presenting feature if there is an impaired access to water, such as during an acute illness or hospitalization.

Diabetes insipidus needs to be ruled out in two circumstances: (i) unexplained hypernatremia, and (ii) polyuria and polydipsia. Whenever a patient has hypernatremia (sodium >145 mEq/L) out of proportion to the history, evaluation of renal concentrating ability is indicated. Many patients who present with hypernatremia show a renal concentrating defect. Less than maximally concentrated urine (<800 mOsm/kg) in the face of hypernatremia associated with signs of dehydration is a sign of a renal concentrating defect, as hypernatremia is a maximal stimulus for ADH release.

Patients with hypernatremia should have a simultaneous measurement of plasma and urine osmolality. Urine osmolality that is less then maximally concentrated (<800 mOsm/kg) in the face of hypernatremia is evidence of a renal concentrating defect. Urine osmolality <300 mOsm/kg is consistent with DI and between 300 and 800 mOsm/kg is consistent with a partial renal concentrating defect. When hypernatremia is associated with a renal concentrating defect, estimation of plasma copeptin helps distinguish central from NDI [79]. Copeptin, the C-terminal segment of the pre-hormone for AVP, can be used as a surrogate marker of ADH as it is easier and more reliable to measure than AVP.

Clinical Manifestations

Hypernatremia results in an efflux of fluid from the intracellular space to the extracellular space to maintain osmotic equilibrium. This leads to transient cerebral dehydration with cell shrinkage. Brain cell volume can acutely decrease by as much as 10–15%, but then quickly adapts [80]. In response to extracellular hyperosmolarity, within 1 h brain cells significantly increase their intracellular content of sodium and potassium, amino acids, and unmeasured organic substances, called idiogenic osmoles. Within 1 week brain cells regain approximately 98% of their water content. If severe hypernatremia develops acutely, the brain may not be able to increase its intracellular solute sufficiently to preserve its volume, and the resulting cellular shrinkage can cause structural changes. Cerebral dehydration from hypernatremia can result in functional changes and a physical separation of the brain from the meninges leading to a rupture of the delicate bridging veins and extra-axial or intracerebral hemorrhages [81]. Venous sinus thrombosis leading to infarction can also develop. Acute hypernatremia has also been shown to cause cerebral demyelinating lesions in both animals and humans (Fig. 5) [82]. Patients with hepatic encephalopathy are at the highest risk for developing demyelinating lesions, especially if hypernatremia is a result of rapid overcorrection of preexisting hyponatremia [83].

Children with hypernatremia are usually agitated and irritable but can progress to lethargy, listlessness, and coma [84]. The degree of central nervous system depression correlates with the severity of hypernatremia. Neurologic examination shows increased tone, nuchal rigidity, and brisk reflexes. Myoclonus, asterixis, and chorea can be present; tonic-clonic and absence seizures have been described. Hyperglycemia is a particularly common consequence of hypernatremia in children. Severe hypernatremia can also result in rhabdomyolysis [85].

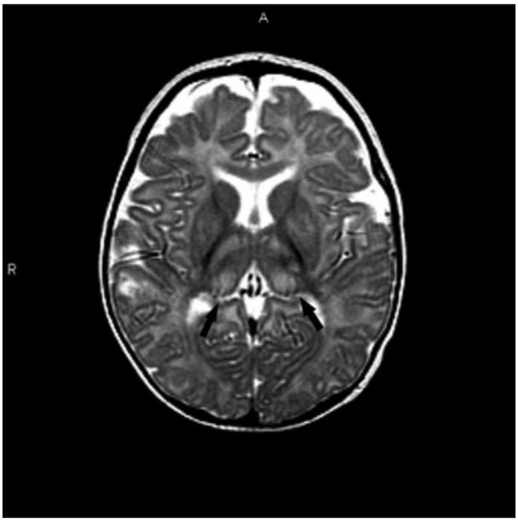

Fig. 5 Extrapontine myelinolysis on CT in a 5-month-old female who presented hypernatremia from central diabetes insipidus and a serum sodium of 195 mEq/L

Treatment

The cornerstone of hypernatremia management is providing adequate free water to correct the serum sodium concentration, but doing this at a rate that limits the risk of brain injury. Hypernatremia is frequently accompanied by volume depletion. If hemodynamic instability is present, fluid resuscitation with an isotonic solution should be instituted to establish normal hemodynamics prior to slowly correcting the free-water deficit (Fig. 6). Following initial volume expansion, the composition of parenteral fluid therapy largely depends on the etiology of the hypernatremia. Patients with sodium overload or a renal concentrating defect require a more hypotonic fluid than patients with volume depletion and intact renal concentrating ability. Oral hydration should be instituted as soon as it can be safely tolerated. Plasma electrolytes should be checked frequently until adequate correction is achieved.

A simple way of estimating the minimum amount of fluid necessary to correct the serum sodium is by the following equation:

$$\text{Free water deficit (ml)} = 4 \times \text{lean body weight (kg)} \times \text{desired change in serum sodium (mEq/l)}$$

Larger amounts of fluid are required depending on the fluid composition. To correct a 3 L free water deficit, approximately 4 L of 0.2% sodium chloride in water or 6 L of 0.45% sodium chloride in water would be required, as they contain approximately 75% and 50% free water, respectively. The calculated deficit does not account for insensible losses or ongoing urinary or gastrointestinal losses. Maintenance fluids, which include replacement of urine volume with hypotonic fluids, are given in addition to the deficit replacement. Glucose containing replacement fluids should be limited as they can result in significant hyperglycemia.

The rate of correction of hypernatremia is largely dependent on the severity of the hypernatremia and the etiology. Due to the brain's relative inability to extrude unmeasured organic substances (idiogenic osmoles), rapid correction

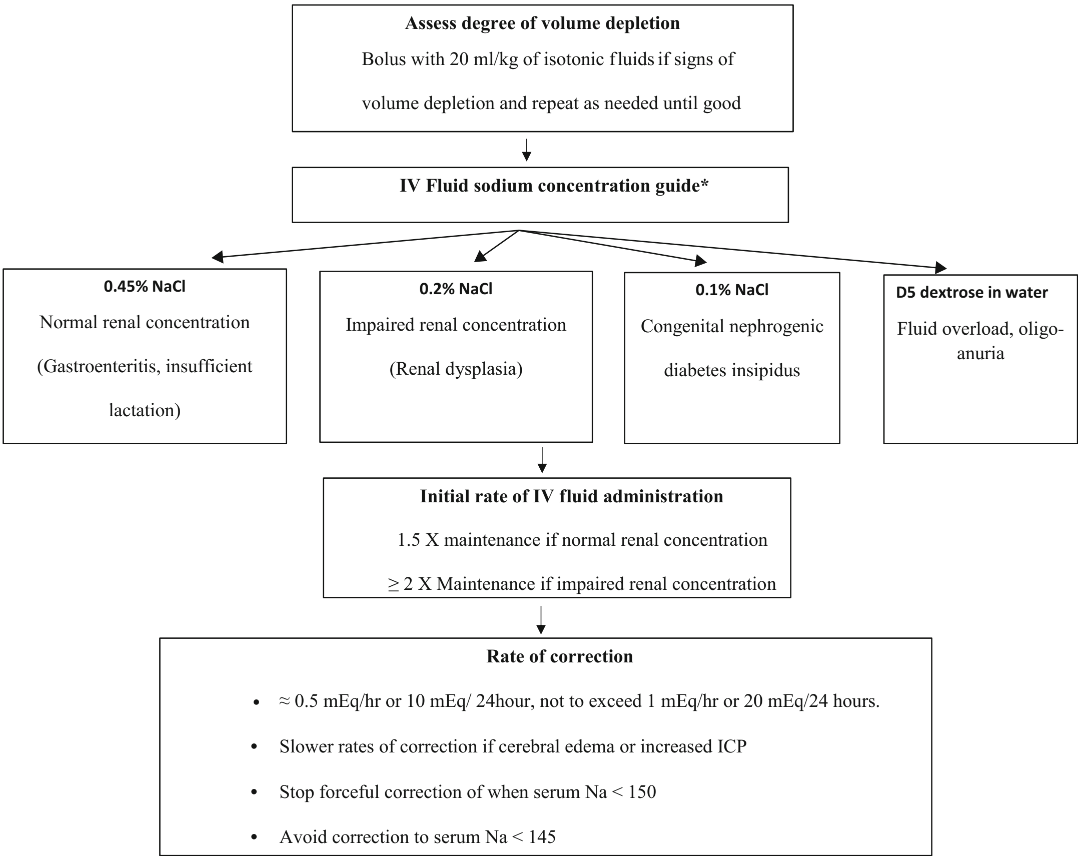
Assess degree of volume depletion
Bolus with 20 ml/kg of isotonic fluids if signs of volume depletion and repeat as needed until good
IV Fluid sodium concentration guide*
0.45% NaCl
Normal renal concentration
(Gastroenteritis, insufficient lactation)
0.2% NaCl
Impaired renal concentration
(Renal dysplasia)
0.1% NaCl
Congenital nephrogenic diabetes insipidus
D5 dextrose in water
Fluid overload, oligo-anuria
Initial rate of IV fluid administration
1.5 X maintenance if normal renal concentration
≥ 2 X Maintenance if impaired renal concentration
Rate of correction
≈ 0.5 mEq/hr or 10 mEq/ 24hour, not to exceed 1 mEq/hr or 20 mEq/24 hours.
Slower rates of correction if cerebral edema or increased ICP
Stop forceful correction of when serum Na < 150
Avoid correction to serum Na < 145

Fig. 6 Management of hypernatremia

of hypernatremia can lead to cerebral edema. While there are no definitive studies that document the optimal rate of correction without developing cerebral edema, correction at a rate not exceeding 1 mEq/h or 15 mEq/24 h is reasonable. In severe hypernatremia (>170 mEq/L), serum sodium should not be corrected to below 150 mEq/L in the first 48–72 h [86]. If the patient is at high risk for developing cerebral edema, such as with head trauma or encephalitis, the rate of correction of hypernatremia should be slower.

It may not be possible to correct hypernatremia with free water alone in patients with severe kidney injury, fluid overload with left ventricular failure, or in cases of severe salt poisoning. When hypernatremia cannot be managed with free water supplementation alone, dialysis may be required [87]. Dialytic therapies provide an option to correct both fluid and electrolyte disorders simultaneously [88].

Seizures occurring during the correction of hypernatremia are not uncommon in children and may be a sign of cerebral edema [89]. Hypotonic fluid infusion should be ceased, and hypertonic saline should be administered when cerebral edema is suspected during the correction of hypernatremia. The presence of signs of intracranial hypertension, such as headache, hypertension, bradycardia, abnormal respiratory pattern, and coma warrants rapid treatment including securing the airway, administration of hypertonic saline, and hyperventilation if herniation seems imminent. In symptomatic children, assessment of progressive cerebral edema by CT head is indicated. Seizures not associated with concomitant cerebral swelling are usually self-limited and not a sign of long-term neurologic sequelae.

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Potassium Disorders: Evaluation and Management

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Marta Suárez-Rivera, Lisa M. Satlin, and Melvin Bonilla-Félix

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Abstract

Potassium, the most abundant intracellular cation, plays a key role in vital cell functions. To ensure somatic growth, children maintain a positive potassium balance. Total body potassium content depends on both dietary intake and its excretion, primarily through the kidneys. The plasma concentration of potassium is a function of the total body potassium content and the internal potassium balance, determined by the relative distribution of potassium between the extracellular and intracellular fluid compartments. Insulin, catecholamines, and acid-base balance play a major role regulating the internal potassium balance. The renal handling of potassium is a complex process that involves filtration, reabsorption, and secretion. These processes are developmentally regulated; thus, the evaluation of children with disorders in potassium balance should always take into consideration the developmental stage of the patient. Hyperkalemia and hypokalemia are common and potentially life-threatening disorders. A careful history, including dietary intake, use of drugs with an effect on potassium metabolism, and evaluation of acid-base balance, is essential to establish an accurate diagnosis. In addition, identifying the cause requires a meticulous assessment of renal excretion of potassium, including estimation of GFR, evaluation of proximal tubular function, and the activity of mineralocorticoids in the distal nephron. The

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treatment should be aimed at correcting the primary cause, when possible. In symptomatic patients, the disturbances in plasma potassium concentration should be corrected promptly, with close monitoring of the electrocardiogram (ECG).

Keywords

Potassium homeostasis · Internal potassium balance · External potassium balance · Hyperkalemia · Hypokalemia

Almost all body content (98%) of potassium is located within cells, where it plays a key role in DNA and protein synthesis, cell growth, and regulating cell volume [1]. The remaining 2% of total body potassium resides in the extracellular fluid, where its concentration is tightly regulated within a narrow range (3.5–5.0 mEq/L in the adult). The internal distribution of potassium between the intra- and extracellular compartments and the external balance between intake and excretion, by the kidney and GI tract, are tightly regulated by multiple complex and efficient mechanisms.

Potassium Homeostasis

Total body potassium content depends on the balance between dietary intake and excretion. In the adult, most (~90%) of the potassium consumed on a daily basis is excreted by the kidney, with only 10% excreted in the feces [2]. A healthy adult maintains a zero potassium balance. Approximately 85% of ingested potassium is reabsorbed by the gut in the adult [3].

Total body potassium content increases from birth to adulthood, with the fastest rate of accumulation during infancy. The increase in total body potassium content correlates with an increase in cell number and potassium concentration with advancing age. Early in life, a positive potassium balance is necessary to ensure somatic growth [4]. This is reflected, in part, in the higher plasma potassium values observed in infants, particularly in preterm neonates [5].

Regulation of Internal Potassium Balance

The regulation of potassium distribution between the intracellular and extracellular compartments is referred to as internal potassium balance. In the adult, the daily dietary intake of potassium of ~1 mEq/kg approaches or exceeds the total potassium present within the extracellular fluid space. This potassium load must be excreted. However, only 50% of an oral or intravenous load of potassium is excreted during the first 4–6 h after its administration [6]. Life-threatening hyperkalemia does not occur because approximately 80% of the absorbed potassium is rapidly translocated from the extracellular space into the intracellular compartment [7]. The buffering capacity of the combined cellular storage reservoirs is capable of sequestering up to approximately 3500 mEq of potassium, which is massive compared with the extracellular pool [2].

To maintain a steep potassium concentration gradient across cell membranes, the body uses a significant amount of energy, provided by the ubiquitous Na-K-ATPase which transports three sodium ions out of and two potassium ions into the cell at the expense of the hydrolysis of adenosine triphosphate (ATP). This unequal cation exchange ratio produces a charge imbalance across the cell membrane, and thus the Na-K-ATPase is defined as an electrogenic pump. Positively charged potassium ions, highly concentrated within the cell, passively leak out of cells down a concentration gradient through ubiquitously expressed potassium-selective channels. A steady state is reached at which the outward movement of positively charged potassium is opposed by the negative cell potential. At this cell equilibrium potential, the net transmembrane flux of potassium is zero.

Regulation of internal potassium balance in the neonate is influenced by developmental stage-specific expression of potassium transporters, including the Na-K-ATPase, as well as channels, receptors, and signal transduction pathways [8, 9]. Thirty to fifty percent of very low-birth weight (birth weight lower than 1500 g) or very preterm (less than 28 weeks postmenstrual age) infants develop nonoliguric hyperkalemia. This is defined as a plasma potassium concentration higher than 6.5 mEq/L within the first 72 hours of life in the presence of normal renal function for age, and normal urine output (>1 ml/kg/h) [10–13]. Although the pathophysiology is not completely understood, it has been proposed that it is the result of immaturity of the mechanisms that regulate internal distribution of potassium, including low rates of Na-K pump activity as well as a limited secretory capacity of the kidney for potassium [11, 12, 14]. Prenatal steroid treatment may prevent nonoliguric hyperkalemia via induction of Na-K-ATPase activity in the fetus [15].

The chemical, physical, and hormonal factors that acutely influence the internal balance of potassium are listed in Table 1 and are discussed below. Potassium uptake into cells is acutely stimulated by insulin, β2-adrenergic agonists, and alkalosis and is blocked by α-adrenergic agonists, acidosis, and hyperosmolality.

Plasma Potassium Concentration

An increase in extracellular potassium concentration favors potassium movement into the cell by the Na-K-ATPase. The concentration of potassium in the extracellular compartment can increase without change in total body potassium content in response to endogenous release of potassium during tissue breakdown (intravascular hemolysis, rhabdomyolysis, and tumor lysis syndrome) and severe exercise, the latter mediated by ATP depletion and opening of ATP-dependent potassium channels.

Table 1 Factors that regulate *internal* potassium balance

Factors	Effect on cell uptake of potassium
Physiologic factors	
Plasma K concentration	
Increase	Increase
Decrease	Decrease
Insulin	Increase
Catecholamines	
α-Agonists	Decrease
β-Agonists	Increase
Pathologic factors	
Acid-base balance	
Acidosis	Decrease
Alkalosis	Increase
Hyperosmolality	Enhances cell efflux
Cell breakdown	Enhances cell efflux

Hormones

Insulin, the most important hormonal regulator of internal potassium balance, stimulates potassium cellular uptake by enhancing Na-K-ATPase activity in the liver, skeletal muscle, adipocytes, and brain. This is independent of its effects on glucose metabolism [16]. Maintenance of fasting plasma potassium concentration within the normal range requires basal insulin secretion [17]. In adults, an increase in plasma potassium greater than 1.0 mEq/L results in a significant increase in peripheral insulin levels, yet a more modest elevation of approximately 0.5 mEq/L has no effect [17–19]. In diabetes, characterized by insulin deficiency, there is a reduction in uptake of potassium by muscle and liver [7].

Catecholamines enhance the cell uptake of potassium via stimulation of Na-K-ATPase activity in skeletal muscle and hepatocytes through β2- adrenergic receptors [20]. The effect of epinephrine on potassium balance in the adult is biphasic and is characterized by an initial increase, followed by a prolonged fall in plasma potassium concentration to a final value below baseline. The initial transient rise in plasma potassium results from α-adrenergic receptor stimulation which causes release of potassium from hepatocytes and impairs cell uptake of potassium [20–23]. β2-receptors stimulate the Na-K pump and thus promote enhanced uptake of potassium by skeletal and cardiac muscle, effects that are inhibited by nonselective β-blockers including propranolol and labetalol [20, 23–27]. The effects of these hormones on the internal potassium balance have been exploited extensively in the treatment of hyperkalemia (Fig. 1 [28]).

Triiodothyronine (T3) promotes Na-K-ATPase-mediated potassium cellular uptake in skeletal muscle [29] primarily as a transcriptional activator of target genes. In addition, there is a nontranscriptional mechanism involving translocation

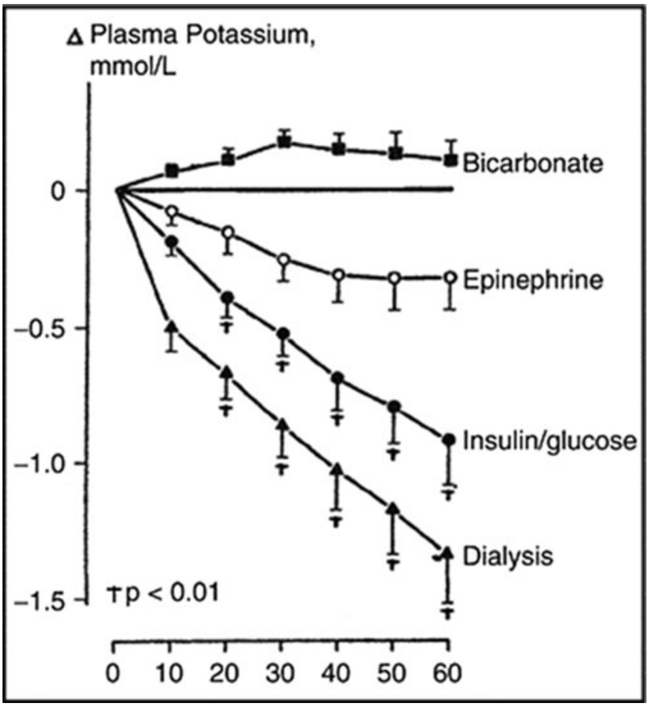

Fig. 1 Changes in plasma potassium concentration (mmol/L) during intravenous infusion of bicarbonate (8.4%), epinephrine, or insulin in glucose and during hemodialysis in adult patients on maintenance hemodialysis (From Blumberg et al., 28)

of Na-K-ATPase to the plasma membrane [30, 31]. The increase in Na-K-ATPase activity in kidney, brain, and lung observed after birth is dependent on normal thyroid status [32].

Acid-Base Balance

Acid-base balance has a direct effect on the internal potassium distribution [33]. While acidemia increases the plasma concentration of potassium by stimulating its exit from the cell, alkalemia stimulates potassium into cells, leading to a decrease in plasma potassium concentration. However, the reciprocal changes in plasma potassium that accompany acute changes in blood pH differ widely among the four major acid-base disorders; metabolic disorders cause greater disturbances in plasma potassium than do those of respiratory origin, and acute changes in pH result in larger changes in plasma potassium than do chronic conditions [34].

Acute metabolic acidosis after administration of an inorganic mineral acid such as hydrochloric acid, which contains an anion (chloride) that does not enter the cells, increases the concentration of potassium in plasma. In this case, the protons of the acid freely enter the cell, where they are neutralized by intracellular buffers, while the impermeant anion stays in the extracellular compartment. To maintain electroneutrality, potassium ions are extruded from the cells. Conversely, if the acidemia is induced by acute organic anion acidosis (lactic acid or acetoacetic acid), there is no change in plasma potassium since the anion of the acid readily enters the cell with the proton, maintaining electroneutrality without the need of potassium to leave the cell [34–36].

In respiratory acid-base disturbances, in which carbon dioxide and carbonic acid readily permeate cell membranes, little transcellular shift of potassium occurs [34]. By contrast, changes in plasma bicarbonate concentration lead to changes in plasma potassium concentration in the opposite direction, independent of the effect on intracellular pH [37]. This explains why during acute respiratory acidosis, a condition characterized by acidemia with an elevated serum bicarbonate, there is minimal increase in plasma potassium, while during acute metabolic acidosis, characterized by acidemia but with a low serum bicarbonate concentration, the increase in plasma potassium concentration is more significant.

Other Factors

A number of other pathologic conditions alter the internal potassium balance. An increase in plasma osmolality causes water to shift out of cells. The consequent increase in intracellular potassium concentration augments the transcellular concentration gradient, favoring its movement to the extracellular compartment. This represents an additional risk in the hyperglycemic diabetic patients, in whom the absence of insulin exacerbates hyperkalemia.

Succinylcholine, a depolarizing paralytic agent and an agonist of nicotinic acetylcholine receptors, which are found predominantly in skeletal myocyte membranes, may lead to efflux of potassium from myocytes into the extracellular fluid under certain pathologic states, including states characterized by upper or lower motor neuron denervation, immobilization, infection, muscle trauma or inflammation, and burn injury [38, 39].

Finally, parenteral administration of cationic amino acids such as lysine, arginine, or epsilon-aminocaproic acid may lead to electroneutral exchange of cell potassium for the cationic amino acid in skeletal myocytes and thus hyperkalemia [40–42].

Regulation of External Potassium Balance

Renal Handling of Potassium

The kidneys are responsible for approximately 90% of potassium excretion. To maintain homeostasis, the goal of the adult, potassium intake should equal excretion. However, the renal response to changes in potassium intake is slow. An increase in dietary potassium intake is matched by a parallel increase in renal potassium excretion within hours, yet maximal rates of potassium excretion are not attained for several days.

Children and adults ingesting an average American diet that contains sodium in excess of potassium excrete urine with a sodium-to-potassium ratio greater than one [43, 44]. Although breast milk and commercially available infant formulas generally provide a sodium-to-potassium ratio of approximately 0.5–0.6, the urinary sodium-to-potassium ratio in the newborn up to 4 months of age generally exceeds 1 and even 2 in newborns <34 weeks of gestational age. This may reflect the greater requirement of potassium over sodium for growth and suggests a relative hyporesponsiveness of the neonatal kidney to mineralocorticoid activity.

The renal handling of potassium is a complex process that involves filtration, reabsorption, and secretion. Plasma potassium is freely filtered at the glomerulus. In the mature kidney, the proximal tubule reabsorbs 60–70% of filtered potassium along with sodium, chloride, and fluid. An additional 25% of filtered potassium is reabsorbed in the thick ascending limb of the loop of Henle, and bidirectional transport (secretion and reabsorption) in the distal nephron [2, 45].

In newborns, potassium clearance is low, even after correction for their low GFR [5, 43, 46]. Infants, like adults, can excrete potassium at a rate that exceeds its GFR, reflecting the capacity for net tubular secretion. However, they are unable to excrete an exogenously administered potassium load as efficiently as the adult [47]. Comparison of the fractional delivery of potassium to the early distal tubule with that present in the final urine reveals that the distal nephron of the young (2-week-old) rat secretes approximately fivefold less potassium than the older (5-week-old) rat [48]. Similarly, clearance studies in saline-expanded dogs also provide indirect evidence of a diminished secretory and enhanced reabsorptive capacity of the distal nephron to potassium early in life [49]. In general, the limited potassium secretory capacity of the immature kidney becomes clinically relevant only under conditions of potassium excess.

Glomerular Filtration

The first step in renal potassium excretion involves filtration through the glomerulus. In adults and older children with CKD, once the GFR is below 25 ml/min/1.73m^2, hyperkalemia may develop due to limited potassium filtration. Almost all premature infants have a GFR lower than 25 ml/min/1.73m^2 during the first week of life, representing a limiting factor for renal potassium excretion.

Proximal Tubule

The concentration of potassium entering the proximal convoluted tubule (PCT) is similar to that of plasma. In mature kidneys, 60–70% of filtered potassium is reabsorbed in the PCT, similar to the absorption rate of sodium and water (Fig. 2) [50–52]. This is not different from the filtered load of potassium (50–60%) reabsorbed along the proximal tubules of suckling (13–15-day-old) rats [48, 53].

Reabsorption of potassium along the early proximal tubule is passive, closely following that of sodium and water [54], and has been proposed to occur by solvent drag, driven by active sodium absorption, and diffusion via the paracellular pathway [55–57]. Potassium diffusion is driven by the lumen-positive transepithelial voltage in the second half of the proximal tubule and the slightly elevated concentrations of potassium in the lumen [58].

In the proximal tubule, as in all other nephron segments discussed below, transepithelial sodium reabsorption requires the coordinated function of apical sodium transporters and the basolateral Na-K-ATPase which actively extrudes sodium from the cell into the interstitium. This maintains a low intracellular sodium concentration, which favors apical sodium entry. Most of the regulation of potassium reabsorption along the proximal tubule can be accounted for by alterations in sodium transport.

Electrogenic sodium-coupled entry of substrates across the luminal cell membrane of the proximal tubule as well as bicarbonate exit across the basolateral cell membrane is driven by the potential differences across the respective cell membranes, which are maintained by potassium flux through potassium channels [59].

Thick Ascending Limb of the Loop of Henle (TALH)

The mature loop of Henle absorbs 25% of filtered potassium through both transcellular and paracellular pathways. The ubiquitous Na-K-ATPase

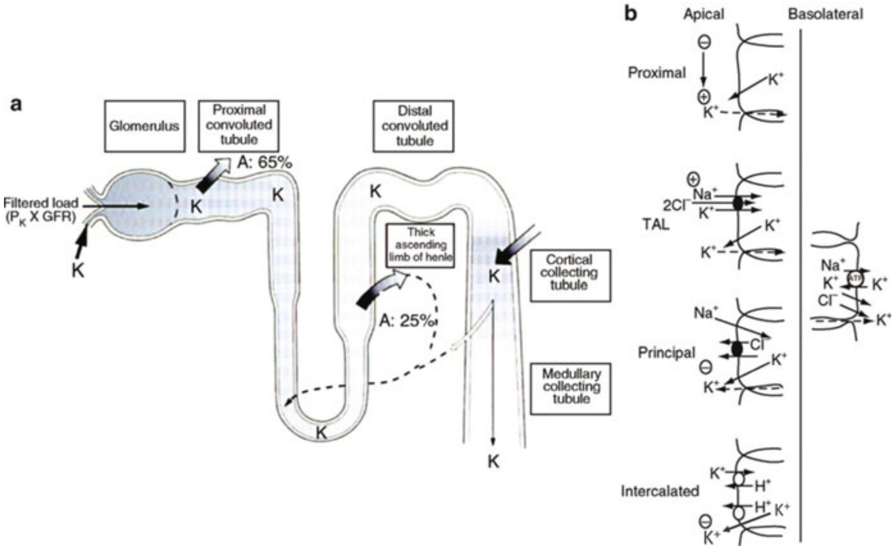
a
Glomerulus
Proximal convoluted tubule
A: 65%
Distal convoluted tubule
Filtered load (P_K X GFR)
K
K
K
K
Thick ascending limb of henle
A: 25%
K
Cortical collecting tubule
Medullary collecting tubule
K
K
b
Apical
Basolateral
Proximal
K⁺
K⁺
TAL
2Cl⁻
Na⁺
K⁺
K⁺
K⁺
Na⁺
K⁺
ATP
Cl⁻
K⁺
K⁺
Na⁺
Cl⁻
Principal
K⁺
K⁺
Intercalated
K⁺
H⁺
H⁺
K⁺
K⁺

Fig. 2 Potassium transport along the nephron. (**a**) The percentages of filtered potassium reabsorbed along the proximal tubule and thick ascending limb of the loop of Henle (TALH) are indicated for the adult (A). Arrows show direction of net potassium transport (reabsorption or secretion). GFR glomerular filtration rate. (**b**) Simplified cell models of potassium transport along the nephron, showing apical transporters unique to discrete nephron segments and cells therein and basolateral transporters which are similar in all nephron segments (From [52])

present in the basolateral membrane maintains a low intracellular sodium concentration which provides the driving force for the apical Na-K-2Cl cotransporter (NKCC2) that translocates a single potassium ion into the cell accompanied by one sodium and two chloride ions (Fig. 2). The diuretics furosemide and bumetanide specifically inhibit NKCC2 and thus block sodium, potassium, and chloride reabsorption at this site.

A high intracellular potassium concentration promotes recycling of some of the absorbed potassium into the lumen via an apical low-conductance potassium channel, known as the renal outer medullary potassium channel (ROMK) since it was originally identified in the thick ascending limb of the rat outer medulla [60–62]. This provides a continuous supply of potassium ions required for the NKCC2 and generates a positive luminal voltage that drives potassium absorption through paracellular pathways, as well as calcium and magnesium. The intracellular potassium leaves the cell through the basolateral membrane, primarily cotransported with chloride or via a potassium channel.

The immature distal nephron receives up to 35% of the potassium-filtered load, in contrast to 15–20% in the adult kidney [48], suggesting that the TALH undergoes a significant postnatal increase in its capacity for reabsorption. This is supported by studies in rats showing a significant increase in the fractional reabsorption of potassium along the TALH, expressed as a percentage of delivered load, between the second and sixth weeks of postnatal life [48].

The mRNA expression of NKCC2 in mice is developmentally regulated. During the early embryonic stage, the signal is undetectable. Expression is first observed in the metanephros by day 14.5 postconception and continues to increase progressively through late gestation. By the end of the first week of postnatal life, the mRNA expression is stronger [63]. In rat kidney, the mRNA encoding NKCC2, absent at birth, is first expressed on postnatal day 8, coincident with completion of nephrogenesis [9]. It increases, at least in medulla, between postnatal days 10 and 40 [64]. Na-K-ATPase activity in the TALH of the neonatal rabbit is only 20% of that measured in the adult when expressed per unit of dry weight [65]. The postnatal increase in pump activity is associated with a parallel increase in expression of medullary Na-K-ATPase mRNA [64].

Distal Nephron

Within the aldosterone-sensitive distal nephron (ASDN) of the fully differentiated kidney, the late distal convoluted tubule (DCT), connecting tubule (CNT), and cortical collecting duct (CCD) are considered to be the primary sites of potassium secretion, which can approach 20% of the filtered load [50, 66–68] (Fig. 2). The DCT secretes a constant small amount of potassium into the urinary fluid [69]. An elevation in plasma potassium concentration inhibits the thiazide-sensitive NaCl cotransporter NCC expressed at the apical membrane of the DCT. The basolateral potassium channel Kir4.1/Kir5.1 acts as a potassium sensor; high potassium intake inhibits Kir4.1/Kir5.1, which leads to inhibition of NCC via the chloride-sensitive WNK pathway [70–72]. This results in increased delivery of sodium into the CD stimulating potassium secretion in this segment. This observation may account for the ability of high potassium diets to lower blood pressure, in a fashion similar to that of thiazide administration [73].

Regulated bidirectional potassium transport occurs in the CNT and CCD, comprised of two major populations of cells. In general, CNT/principal cells reabsorb sodium and secrete potassium, whereas intercalated cells regulate acid-base balance but can reabsorb potassium in response to dietary potassium restriction or metabolic acidosis (Fig. 2) [74, 75]. Thus, the direction and magnitude of net potassium transport in these segments depend on the metabolic needs of the organism and reflect the balance of potassium secretion and absorption.

Potassium Secretion

Potassium secretion in the distal nephron is critically dependent on the reabsorption of sodium delivered to these segments. In the principal cells, sodium is absorbed via the luminal epithelial Na channel (ENaC) and then diffuses into the interstitium via the basolateral Na-K-ATPase (Fig. 2). The removal of the positive charge (Na+) from the lumen and accumulation of potassium within the cell creates a favorable electrochemical gradient for potassium secretion through apical potassium-selective channels. Basolateral potassium channels in these cells provide a route for intracellular potassium ions to recycle back into the interstitium, thereby maintaining the efficiency of the Na-K pump. Any factor that increases the electrochemical gradient across the apical membrane or increases the apical potassium permeability will promote potassium secretion. An apical electroneutral potassium chloride cotransporter has also been functionally identified in the CCD [76, 77].

Two major apical potassium-selective channels have been identified in the distal nephron: the small-conductance secretory potassium (SK) channel and the high-conductance BK channel [78].

The SK channel, restricted to the CNT/principal cell, mediates baseline constitutive potassium secretion [79, 80]. ROMK is considered to constitute the SK channel since there is absence of SK channel activity in apical membranes from both TALH and collecting duct in ROMK null mice [81].

The BK channel intercalated cells is considered to mediate flow-stimulated potassium secretion [82, 83]. The BK channel, rarely open at the physiologic conditions, can be activated by cell depolarization, membrane stretch, and increases in intracellular calcium ion concentration elicited by increases in urinary flow rate [84–87]. The role of the BK channel in flow-stimulated urinary potassium secretion has been confirmed in mouse models [88, 89]. The BK channel assumes great importance in regulating potassium homeostasis under conditions where SK/ROMK channel-mediated potassium secretion is limited.

The “with-no-lysine” kinases, or WNK kinases, comprise a family of serine/threonine kinases uniquely characterized by anomalous placement of the catalytic lysine crucial for binding to ATP. They act as molecular switches that direct differential effects on downstream ion channels, transporters, and have an essential role in sodium and potassium regulation [90]. WNKs phosphorylate and thereby activate the thiazide-sensitive NCC [91]. WNK1, WNK3, and WNK4 are all expressed in the kidney. Intracellular chloride concentration plays a major role modulating WNK activity: Chloride binds to the catalytic domain of WNKs, blocking its autophosphorylation and kinase activity. However, WNK kinases are chloride but not potassium sensitive. It seems that the regulation of NCC by potassium involves modulation of intracellular chloride concentration [92]. Kir4.1 regulates NCC expression by the modulation of the Cl−sensitive with-no-lysine kinase–SPAK pathway.

Potassium Absorption

In conditions of potassium depletion or metabolic acidosis, the distal nephron may become a potassium-absorbing segment. Potassium is actively absorbed in exchange for protons through the H-K-ATPase, localized to the apical membrane of alpha intercalated cells [74, 93–95]. Two isoforms of the H-K-ATPase are found in the kidney: The gastric isoform, HKAg, is normally found in gastric parietal cells and is responsible for acid secretion into the lumen whereas the colonic HKAc isoform is a structurally related H-K-ATPase found in distal colon that mediates active potassium reabsorption [94]. Expression of the apical gastric-like H-K-ATPase in the rat and rabbit intercalated cell is increased in response to dietary potassium restriction and metabolic acidosis [74, 93, 96, 97]. A reduction in potassium intake leads to a fall in potassium secretion by the distal nephron within 5–7 days in rat [98]. This adaptation is associated with a decrease in the number of apical SK/ROMK [99] and BK [100] channels and stimulation of H-K-ATPase-mediated potassium reabsorption in intercalated cells in the distal nephron [101]. Stimulation of luminal H-K-ATPase activity in intercalated cells results not only in potassium retention but also in urinary acidification and metabolic alkalosis.

Developmental Regulation of Distal Potassium Transport

Potassium secretion measured in isolated microperfused CCD from neonatal rabbits at physiological flow rates is absent at birth. It first becomes evident at 4 weeks of age and increases abruptly after that, reaching maturity by 6 weeks of age [68]. Patch clamp analysis in maturing rabbit CCD showed a clear developmental increase in

ROMK channel activity between 2 and 5 weeks of age, consistent with data from isolated perfused CCDs at low flow rates. This suggests that the appearance of baseline potassium secretion in the CCD is the result of the development of SK/ROMK channels [102]. Flow-stimulated potassium secretion in the rabbit appears by 5 weeks of postnatal life, parallel with the appearance of BK channel expression.

Renal Response to Dietary Potassium

It has been shown that changes in dietary potassium can induce kaliuresis before an increase in plasma potassium level or a change in aldosterone concentration is detectable. This data suggests a gut-sensing mechanism that directly stimulates renal potassium excretion, not mediated by aldosterone [99]. After ingestion of a potassium-containing meal, an increase in the potassium concentration in the splanchnic circulation stimulates local sensors that can directly induce a kaliuretic response. This process, known as the feed forward mechanism, does not require an increase in plasma potassium concentration and is not regulated by aldosterone (205). Evidence for a potassium sensor in the hepatorenal system arises from experiments showing that an infusion of KCl directly into the hepatoportal circulation increases urinary potassium excretion in the absence of any elevation in plasma potassium. This effect is abolished by bumetanide, suggesting the presence of an isoform of the Na-K-2Cl cotransporter, NKCC1, in the hepatoportal system that mediates a signal transmission to the kidneys with consequent kaliuresis [103, 104]. The inverse is observed during conditions of chronic dietary potassium restriction. Before hypokalemia develops, the renal excretion of potassium is reduced, probably mediated by sensors in the gastric or hepatoportal circulation that in some way inactivates ROMK [105].

Hormones

Aldosterone stimulates sodium reabsorption and potassium secretion in principal cells of the fully differentiated ASDN [106, 107]. Among the cellular and molecular effects of an increase in circulating levels of aldosterone are increases in density of ENaC channels, achieved by the recruitment to and retention of intracellular channels at the apical membrane, de novo synthesis of ENaC subunits, and activation of preexistent channels, as well as stimulation of Na-K-ATPase activity by translocation of preformed transporters to the membrane and translation of new sodium pump subunits [108–113].

Plasma aldosterone concentrations in premature infants and newborns are higher than those measured in the adult [43, 114]. Yet, clearance studies in fetal and newborn animals demonstrate a relative insensitivity of the immature kidney to the hormone [43, 115] [43, 115–117]. The density of aldosterone binding sites, receptor affinity, and degree of nuclear binding of hormone receptor are believed to be similar in mature and immature rats [115].

Circadian Control of Renal Potassium Excretion

Renal potassium excretion varies during the 24-h day in the adult [118, 119]. Although some of this variation can be attributed to timing of meals, a pattern of low rates of potassium excretion at night and in the morning and high rates in the afternoon is evident, independent of feeding, drinking, and activity [118, 119]. The distal nephron expresses core clock genes such as period circadian regulator 1 gene (PER1), a protein coding gene that is part of the clock gene network. ENaC, aquaporin 2, and vasopressin receptors have marked circadian rhythms, which could influence indirectly the excretion of potassium [120, 121]. Moreover, recent data from space flight simulation suggest that there is also an infradian rhythmicity to aldosterone secretion [122]. It is unknown whether a circadian cycle of urinary potassium excretion prevails in infancy.

Contribution of the Gastrointestinal Tract

Up to 90% of the dietary potassium is absorbed in the gastrointestinal tract. The colon is considered to be the main target for regulation of intestinal potassium excretion [123]. Potassium transport in the colon represents the balance of secretion and absorption [124]. Under baseline conditions, net potassium secretion predominates over absorption in the adult, whereas the neonatal gut is poised for net potassium absorption [123].

Potassium secretion requires potassium uptake by the Na-K-ATPase and NKCC located in the basolateral membrane of colonocytes; potassium is then secreted across the apical membrane through potassium channels, including a calcium-activated BK channel similar to that found in the distal nephron [125–129]. Potassium absorption is mediated by two H-K-ATPases localized to the apical membrane of the distal colon [130].

Stool potassium content can be enhanced by any factor that increases colonic secretion, including aldosterone, epinephrine, and prostaglandins [7, 131, 132]. Indomethacin and dietary potassium restriction reduce potassium secretion by inhibiting the basolateral transporters and apical potassium channels. Diarrheal illnesses are typically associated with hypokalemia, presumably due to the presence of nonreabsorbed anions, an enhanced electro-chemical gradient established by active chloride secretion, and secondary hyperaldosteronism due to volume contraction [133].

Potassium adaptation in the colon is demonstrated by increased fecal potassium secretion after potassium loading and in the face of renal insufficiency. Fecal potassium excretion may increase substantially to account for 30–50% of potassium excretion in patients with severe chronic kidney disease [7, 134–136]. The enhanced colonic potassium secretion characteristic of renal failure requires induction and/or activation of apical BK channels in surface colonic epithelial cells [137].

Experimental data demonstrate that the immature intestine absorbs potassium more avidly than the adult. Intestinal potassium absorption measured after intragastric administration of KCl is higher in infant than in adult rats. In addition, rates of total cumulative Rb uptake – a marker of potassium transport – are higher in isolated distal colons from infant rats, suggesting increased potassium absorption in the distal colon of immature animals [138]. The higher rates of intestinal potassium absorption in infant animals are the consequence of lower activity of the basolateral Na-K-ATPase and increased activity of apical potassium-absorptive pumps, such as H-K-ATPase and ouabain-sensitive Na-independent K-ATPase. This results in lower intracellular potassium concentration promoting net intestinal absorption in the neonate, in contrast to net secretion in the adult colon. This will contribute to a state of total body potassium retention and higher extracellular concentration in the neonatal period [123, 127, 139].

Disorders in Potassium Balance

Hyperkalemia

Hyperkalemia is a common and potentially life-threatening electrolyte disorder, defined as a plasma potassium concentration higher than 5 mEq/L in the adult. Before the diagnosis of hyperkalemia is established, pseudohyperkalemia, which results from release of intracellular potassium during phlebotomy or specimen processing, must be ruled out. Most laboratories will alert a healthcare provider as to the presence of hemolysis. However, in most instances, assessment of the degree of hemolysis in a blood sample is subjective, based on visual inspection of the color of the plasma. Therefore, as a general rule, all samples obtained by a heel stick, from fingertip or after prolonged use of tourniquet that show an elevated potassium level, should be considered a hemolyzed sample and must not be used to make therapeutic decisions.

Altered Internal Balance

Hyperkalemia due to an altered internal balance should be suspected if there are suggestive factors, such as metabolic acidosis and/or poorly controlled diabetes mellitus (see Table 1). Treatment in these cases should focus on correcting the underlying abnormality.

Altered External Balance

Hyperkalemia due to an altered external balance may be due to excessive potassium intake, impaired renal potassium secretion, or a combination of both. With normal kidney function, even a very high potassium intake should not lead to hyperkalemia, due to the rapid translocation of potassium into cells, unless administered as an acute bolus [140].

Commonly used tools to assess renal potassium secretion include the fractional excretion of potassium (FEK), the ratio of urinary potassium to sodium, and the transtubular potassium gradient (TTKG). The TTKG provides an indirect measure of the mineralocorticoid activity in the ASDN and is calculated using the equation:

$$\mathrm{TTKG} = \frac{(\mathrm{Urine}\ [K])/(\mathrm{Plasma}\ [K])}{(\mathrm{Urine\ Osmolality})/(\mathrm{Plasma\ Osmolality})}$$

where [K] equals the potassium concentration in mEq/L [141–143]. Measurements of TTKG have been reported to be lower in 27- than 30-week gestational age preterm infants followed over the first 5 weeks of postnatal life [144]. A TTKG value of 4.1 corresponded to the 3rd percentile for TTKG results and 13.1 to the 97th percentile [144]. Accordingly, in hyperkalemia, a TTKG value below 4.1 would be inappropriate and indicate low mineralocorticoid activity, suggesting impaired renal potassium secretion. Conversely, a TTKG value above 13.1 is consistent with high mineralocorticoid activity and would thus suggest excessive potassium intake as the underlying etiology of the hyperkalemia. However, although TTKG may be useful in the evaluation of children with potassium disturbances, during recent years several pitfalls have been identified. In states where the rate of excretion of urea deviates appreciably from normal, TTKG may overestimate the true renal K excretion. In addition, adequate urine sodium concentration (> 25 mEq/L) and a urine that is hypertonic compared to plasma are required to be able to use TTKG (303, 304). In preterm neonates, TTKG is low during the early neonatal period and does not correlate with plasma aldosterone levels, consistent with the low-tubular K excretion and mineralocorticoid resistance. This finding is probably physiologic or related to the presence of associated medical conditions such as the use of mechanical ventilation [145]. Therefore, TTKG should not be used in premature infants as the interpretation of this test is troublesome since developmental stage-specific limitations in renal potassium handling and urine concentration may confound the results.

In the neonatal period, hyperkalemia is defined as a plasma potassium concentration > 6 mEq/L in full-term neonates and of >6.5 mEq/L in premature infants. In very premature infants (postmenopausal age < 32 weeks), normal plasma potassium concentration could be up to 6.7 mEq/L during the 1st week of life [11, 146].

Causes of Hyperkalemia

The most common causes of hyperkalemia in infants and children are shown in Table 2. Neonates are more susceptible to effects of some medications, which can limit the already compromised Na-K-ATPase activity, the ability to shift K^+ into the cell, and the tubular response to mineralocorticoids. These include succinylcholine, digoxin overdose, and β2 adrenergic blockers causing an efflux of potassium from the cell and inhibition of Na-K-ATPase, respectively [38, 39, 147]. Other medications can induce hyperkalemia by acting on the angiotensin-aldosterone axis including spironolactone,

Table 2 Causes of hyperkalemia in infants and children

Causes	Examples
Pseudohyperkalemia	Heelstick Finger tip Prolonged tourniquet
Increased potassium load	Diet Oral supplements (KCl, KCitrate, KPhosphate) Intravenous fluids/TPN Blood transfusions Hemorrhages (GI, IVH-in newborns) Hemolysis Rhabdomyolysis Tissue necrosis
Decreased renal excretion	AKI and CKD Hypoaldosteronism/ pseudohypoaldosteronism Urinary obstruction
Drugs	SpironolactoneAmilorideTrimethoprim Nonsteroidal anti-inflammatory drugs Calcineurin inhibitors Heparin Succinylcholine Penicillin G
Redistribution	Acidosis Nonoliguric hyperkalemia of the neonate

indomethacin, and angiotensin converting enzyme inhibitors. Inhibition of ENaC by amiloride and trimethoprim reduces the sodium excretion and potassium excretion [148].

Endogenous potassium load can result from tissue breakdown, gastrointestinal bleeding, and tissue necrosis. Careful attention should also be given to excess oral or intravenous potassium supplementation/nutrition, medications with potassium salts, such as penicillin, or blood transfusions. Acute kidney injury and CKD also result in impairment of urinary potassium excretion. Decreased aldosterone activity, characterized by variable degrees of metabolic acidosis, renal salt wasting, and hyperkalemia, results from a deficiency in mineralocorticoids or resistance to its effects. Aldosterone synthase deficiency is a rare autosomal recessive disease characterized by defective biosynthesis of aldosterone that results in isolated hypoaldosteronism. Patients exhibit salt wasting, recurrent dehydration, hyperkalemia, and failure to thrive. Neonatal screening using 17-hydroxyprogesterone measurement identifies a defect in 21-hydroxylase but fails to detect aldosterone synthase deficiency, a diagnosis which may be missed until the patient presents with a salt-wasting crisis [149, 150]. Pseudohypoaldosteronism is a rare hereditary disorder that results from end-organ resistance to aldosterone, characterized by high plasma aldosterone and renin concentrations. Type I (PHA1) is the result of mutations in the mineralocorticoid receptor (PHA1a or renal PHA) or mutations in the sodium epithelial channel (PHA1b or systemic PHA) [151]. Pseudohyperaldosteronism Type 2 (PHA II; familial hyperkalemic hypertension, FHHt, Gordon Syndrome), a hereditary disorder characterized by hyperkalemia, metabolic acidosis, and hypertension sensitive to thiazide diuretics, results from mutations in the genes encoding WNK1, WNK4, and the ubiquitin-ligase elements Kelch-like 3 and Cullin 3, which are involved in the ubiquitination and degradation of WNK4 (157–161). Hypertension usually develops in late childhood, but the electrolyte disturbances – hyperkalemic acidosis – may be present at birth [152].

Evaluation of Hyperkalemia

The evaluation of hyperkalemia starts by identifying life-threatening signs. Initial ECG changes are prolonged PR interval and peaked T waves, followed by an absence of P wave, widened QRS with S-T depression, and persistent peaked T waves. The QRS continues to widen with higher plasma potassium concentration until ventricular fibrillation develops. Symptoms, such as muscle weakness and hypotension, are rarely observed. One should also assess the blood pressure, as well as volume status and the genitalia since infants with congenital adrenal hyperplasia can present with salt wasting, severe dehydration, hypotension, and virilization.

The laboratory evaluation must include plasma concentrations of sodium, chloride, bicarbonate, creatinine, and a complete blood count. If the GFR is normal and no offending agent can be identified, hypo- or pseudohypoaldosteronism should be ruled out. In these patients, hyperkalemia will be accompanied by hyponatremia and metabolic acidosis. TTKG, with the previously discussed limitations, could assist to discriminate between renal and extrarenal causes. In children with hyperkalemia due to a nonrenal cause, the urinary potassium excretion is very high and therefore the TTKG is high (> 11). In newborns with hypo- and pseudohypoaldosteronism, the TTKG is very low (1.4–4.1) [144]. If an adrenal etiology is suspected, one should measure serum levels of aldosterone, renin, and cortisol.

Treatment of Hyperkalemia

Treatment varies depending on the etiology of hyperkalemia. The intake of potassium should be reduced if indicated. In those with impaired potassium secretion, the cause should be identified. If clinical evidence of volume depletion is present, hyperkalemia may simply reflect inadequate distal sodium delivery and treatment should be aimed at volume expansion.

Therapy targets for hyperkalemia are to prevent arrhythmias and to decrease the plasma concentration of potassium by stimulating a shift into the cell or increasing excretion. If the ECG shows changes consistent with hyperkalemia, calcium gluconate 10% should be administered at a dose of 0.5–1 ml/kg over 5–15 minutes (Fig. 3). The effect stabilizing the cardiac membrane is evident in 1–3 minutes and lasts for 30–60 minutes. The dose may be repeated after 5 minutes if ECG changes persist [153].

Insulin/glucose administration and β2-adrenoreceptor agonists enhance transcellular potassium shifts into the cell. The administration of glucose alone (to induce endogenous insulin release) or glucose plus insulin is efficacious, although patients must be monitored for disturbances in blood glucose, especially in neonates [154–156]. Insulin can be administered at a dose of 0.1–0.6 units/kg/h with glucose infusion of 0.5–1 g/kg/h [157]. The onset of action is approximately15 minutes and lasts few hours. Administration of albuterol/salbutamol via nebulizer has been used to treat acute hyperkalemia in neonates, children, and adults [158–160]. A single dose of nebulized albuterol can lower serum potassium by as much as 0.5 mEq/L [161, 162]. β agonists have a rapid onset of action. It can be administered by a nebulizer at a dose of 400 micrograms (newborns), 2.5 mg (in small children), and up to 5–20 mg in older children and adults [159]. This decreases the plasma concentration of potassium by 0.5–1 mEq/L. Intravenous albuterol at a dose of 4–5 μg/kg, given as an intravenous slow bolus over 5 min, decreases plasma potassium concentration by 0.9–1.5 mEql/L [162].

In the presence of metabolic acidosis, sodium bicarbonate infusion can also promote potassium shifting into the cell. Because it is hyperosmolar and the potential association with intraventricular hemorrhages in premature infants, it should be used very judiciously during the neonatal period [163, 164]. In this case, we recommend a dose of 1 mEq/kg diluted 1:4 in sterile water, administered over 30–60 minutes. The efficacy is questionable in the absence of acidosis. To remove body potassium stores, if the patient is making urine, one could use loop or thiazide diuretics. For severe, unremitting hyperkalemia, dialysis is required. For the removal of potassium from the

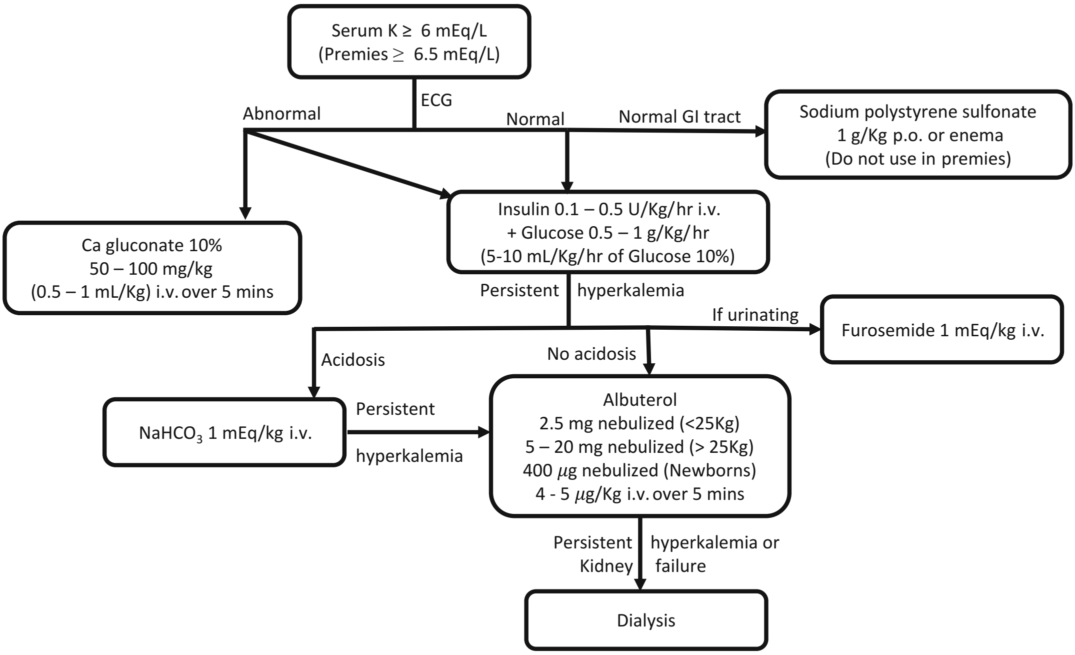
Serum K ≥ 6 mEq/L
(Premies ≥ 6.5 mEq/L)
ECG
Abnormal
Normal
Normal GI tract
Sodium polystyrene sulfonate
1 g/Kg p.o. or enema
(Do not use in premies)
Ca gluconate 10%
50 – 100 mg/kg
(0.5 – 1 mL/Kg) i.v. over 5 mins
Insulin 0.1 – 0.5 U/Kg/hr i.v.
+ Glucose 0.5 – 1 g/Kg/hr
(5-10 mL/Kg/hr of Glucose 10%)
Persistent hyperkalemia
If urinating
Furosemide 1 mEq/kg i.v.
Acidosis
No acidosis
NaHCO3 1 mEq/kg i.v.
Persistent hyperkalemia
Albuterol
2.5 mg nebulized (<25Kg)
5 – 20 mg nebulized (> 25Kg)
400 μg nebulized (Newborns)
4 - 5 μg/Kg i.v. over 5 mins
Persistent hyperkalemia or
Kidney failure
Dialysis

Fig. 3 Treatment of hyperkalemia in infants and children. Besides dialysis, sodium polystyrene sulfonate is the only treatment that decreases total body potassium. It can be used at any point if the patient has a normal gastrointestinal (GI) tract. It should be avoided in premature infants. The order of insulin/glucose and albuterol can be changed, according to availability and clinical situation. If the patient is urinating, furosemide can be added at any point to enhance renal potassium excretion

body, resins such as sodium polystyrene 1 g/Kg orally or as enemas can be used. Its efficacy is questionable in neonates [165]. Moreover, because it carries a risk of intestinal perforation and obstruction, it is contraindicated in infants with necrotizing enterocolitis or very premature infants [166]. Newer and more efficacious resins – patiromer and sodium zirconium cyclosilicate – are available for adults, but use in children is currently under investigation [167].

Hypokalemia

Hypokalemia is defined as a plasma potassium concentration lower than 3.5 mEq/L. Since potassium is primarily an intracellular cation, the plasma concentration is not an accurate estimate of total body potassium. Under normal conditions, changes in intracellular potassium concentration parallel serum potassium. Factors that regulate internal potassium distribution may affect this relationship [168]. Cardiac arrhythmias, muscle weakness, and even paralysis are major morbidities associated with severe hypokalemia that requires emergency treatment.

Altered Internal Balance

Hypokalemia due to an altered internal balance should be suspected if there are suggestive factors, such as metabolic alkalosis or evidence of increased insulin activity (Table 1). Treatment in these cases should focus on correcting the underlying abnormality.

Altered External Balance

Hypokalemia due to an altered external balance can be either from excessive potassium loss from the intestine or kidney, insufficient intake, or a combination of these factors. As with hyperkalemia, assessment of the urinary potassium excretion with TTKG may provide useful information to identify the etiology. A low TTKG suggests renal potassium conservation and thus points toward intestinal losses and/or insufficient intake. Conversely, a high TTKG points to renal potassium wasting. In the context of volume depletion, this finding would likely reflect aldosterone-mediated attempts to maintain extracellular volume at the expense of potassium homoeostasis, and volume repletion is the appropriate treatment. Hypokalemia with high TTKG in the presence of hypervolemia and hypertension suggests primary hyperaldosteronism or pseudohyperaldosteronism as seen in some inherited disorders, such as Liddle syndrome or apparent mineralocorticoid excess (Table 3). Pseudohyperaldosteronism can also be acquired, as occurs with licorice abuse [169]. Treatment should focus on the underlying defect. In the short term, or if the underlying problem cannot be targeted, distal potassium secretion can be reduced either by administration of inhibitors of ENaC (amiloride) and/or mineralocorticoid receptor (spironolactone or eplerenone).

Table 3 Causes of hypokalemia in infants and children

Causes	Examples
Poor intake	Prolonged starvation Insufficient support in TPN
Increased renal losses	Mineralocorticoid excess Bartter syndrome Gitelman syndrome Renal tubular acidosis Magnesium deficiency Diabetic ketoacidosis
Gastrointestinal losses	Vomiting Diarrhea GI fistulas Ostomies
Drugs	Amphotericin B Aminoglycosides Thiazides Loop diuretics Corticosteroids
Redistribution (internal shifts)	Alkalosis Insulin Theophylline/caffeine Thyrotoxicosis β agonists, epinephrine

Causes of Hypokalemia

The most common causes of hypokalemia in children are shown in Table 3. Gastrointestinal disturbances such as vomiting, diarrhea, or unreplaced electrolyte losses from nasogastric or intestinal drainage may lead to hypokalemia. Infants with necrotizing enterocolitis or congenital intestinal anomalies requiring surgery of the intestinal tract are at particular risk. Renal potassium losses often result from the use of medications including diuretics, such as furosemide and thiazides or the antifungal amphotericin B (forms pores in cell membrane that allows K^+ to leak out of the cell). The use of the aldosterone inhibitor spironolactone as a potassium-sparing agent may prevent hypokalemia in patients receiving diuretics. Amphotericin B lipid complex is associated with a lower incidence of hypokalemia [170]. Hereditary disorders associated with alkalosis (Bartter and Gitelman syndromes) or acidosis (renal tubular acidosis) cause hypokalemia during the neonatal period. Bartter syndrome, characterized by large sodium losses, is associated with hypokalemic alkalosis and polyhydramnios. Gitelman syndrome usually presents later in life, although hypokalemia has been reported in the newborn period [171]. Renal tubular acidosis, type 1 and 2, often features hypokalemia along with metabolic acidosis. In the Fanconi syndrome, hypokalemia is associated with acidosis, hypophosphatemia, and hypouricemia; the urine is usually dilute with glycosuria, aminoaciduria, and tubular proteinuria. The presence of hypertension in a child with mild hypokalemic alkalosis should prompt the diagnosis of glucocorticoid-remediable aldosteronism, especially if there is familial history of premature cerebrovascular events [172, 173].

Evaluation of Hypokalemia

Severe hypokalemia can cause cardiac arrhythmias, paralytic ileus, muscle weakness, and lethargy. Changes in the ECG include flattened T waves, prolongation of the QT interval, and the appearance of U waves. The primary cause of hypokalemia should be identified and treated accordingly. To this end, a random assessment of the urinary potassium in patients with disturbances of potassium balance is inadequate to assess if the etiology resides in the kidney. Alternatively, given the limitations previously discussed, TTKG may be a helpful tool.

In patients with hypokalemia, the serum concentration of magnesium should be measured as hypokalemia might worsen and become refractory to treatment in the presence of hypomagnesemia [73]. If hypokalemia is associated with polyuria, which is common, intravascular volume should be promptly restored. The rate and route for replacement of potassium depends on the presence of symptoms or changes in ECG.

Treatment of Hypokalemia

The safest treatment of mild asymptomatic hypokalemia is via the oral/enteral route using potassium salts in a dose of 1–2 mEq/kg/day (Fig. 4). In the presence of alkalosis, potassium should be given as KCl to replenish chloride [174]. Moderate to severe (<2.5 mEq/L) symptomatic hypokalemia, or hypokalemia in a patient with gastrointestinal complications who cannot tolerate oral salts, should be treated using intravenous preparations of potassium. The addition of KCl to intravenous fluid (not to exceed 40 mEq/L) can be sufficient in cases of moderate hypokalemia. Higher concentrations (60–80 mEq/L) are required if the patient fails to respond but should be given through a central vein under continuous ECG monitoring. Rapid administration of KCl can lead to life-threatening arrhythmias. In cases of severe (<2 mEq/L) or symptomatic hypokalemia, KCl diluted in normal saline can be carefully given intravenously in doses up to 0.5–1 mEq/kg over 1–2 hours [175]. Continuous ECG monitoring is required. Potassium salts should not be diluted in dextrose-containing solutions as it provokes insulin secretion and cellular potassium uptake, lowering plasma potassium concentration even further [176].

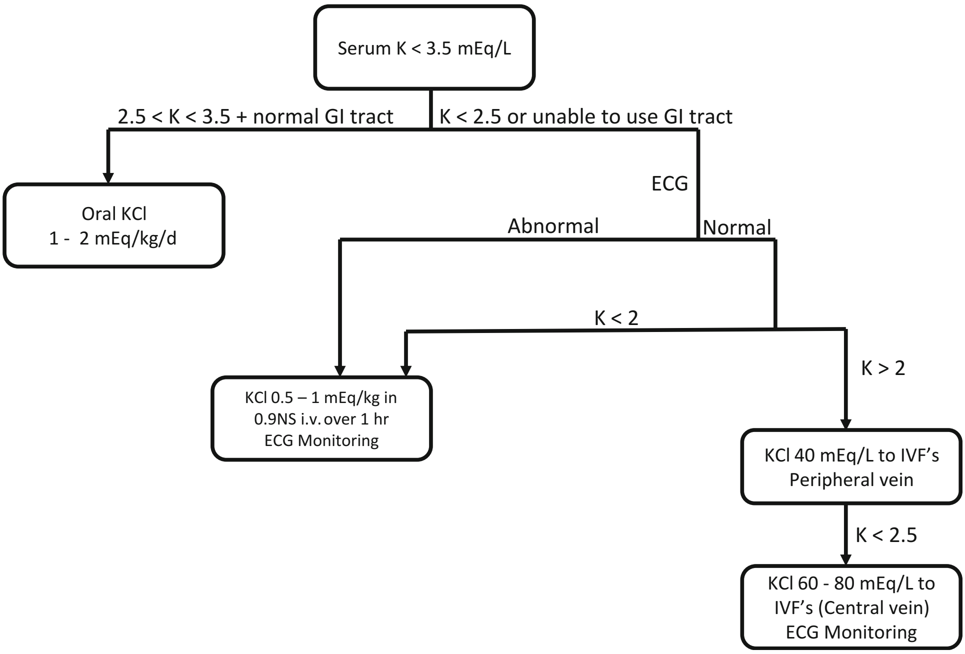

Fig. 4 Treatment of hypokalemia in infants and children. Provided that the patient has a normal GI tract, oral potassium salts should be the initial step in the treatment of mild hypokalemia (K > 2.5 mEq/L). Treatment of severe hypokalemia (K < 2 mEq/L) should be done under continuous ECG monitoring, preferably in a critical care unit. When administering potassium intravenously, always dilute in 0.9NS and not in dextrose as dextrose stimulates insulin secretion and translocation of potassium into the cell, worsening hypokalemia

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Clinical Use of Diuretics

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Paul Goodyer, Marina Mir, and Sam Shemie

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Dosing and Pharmacokinetic Properties of Commonly Used Diuretics in Pediatrics (Table 2) 1164

References 1164

Abstract

Diuretics are among the most commonly prescribed medications for patients with kidney disease. Most diuretics exert their action by preventing sodium re-absorption from the lumen of the nephron. Since cells must maintain electrochemical neutrality, the direct drug effect on sodium and other electrolytes must be counterbalanced, and must be accounted for as potential side effects. The current chapter will review the specific actions of available diuretics and their applications in clinical settings.

Keywords

Diuretics · Dosing · Pharmacokinetics

Medicinal substances increasing the flow of urine have been recognized in medical writings for over 2000 years. In this chapter we review the physiology of commonly used diuretics on salt and water transport in specific nephron segments and then discuss practical applications of diuretics in clinical pediatric settings. In addition to the importance of diuretics in treating various states of fluid overload, we review additional indications for diuretics in special conditions ranging from kidney stones, to glaucoma, to anxiety.

Introduction

The English word, diuretic, derives from the ancient Greek word, *diouretikos* – referring to any substance that promotes urinary flow. Long lists of plants containing natural diuretics were recognized by Discorides (De Materia Medica) and Pliny the Elder (Naturalis Historia) in the first century [1]. Plants with diuretic properties have been alluded to in Traditional Chinese and Indian medicine writings for over 2000 years [2]. Interestingly, the common dandelion was termed, in Old French, a "Pissenlit" – making reference to bedwetters. However, most naturally occurring diuretics are relatively mild and are of marginal benefit in treating severe edematous states in humans. Clare et al estimate that the diuresis caused by ingestion of an extract of dandelion greens equivalent to an oral dose of 1 g/kg in humans has a diuretic effect equal to a 1 mg/kg dose of furosemide [3] – a potency of about 1:1000.

During the early part of the twentieth century, Western physicians treated edema with mercurials (which proved to be toxic) and various osmotic diuretics such as mannitol and sucrose. However, it was not until 1937 that Southworth made the seminal observation that would lead to the development of the first modern diuretics. He noted a significant diuresis in patients receiving the antibiotic, sulfanilamide. By 1949, Schwartz had used this drug to treat congestive heart failure [4]. Over the ensuing years, Beyer began testing sulfonilamide derivatives for their diuretic properties in animals and finally developed chlorthiazide, the first of the thiazide class of diuretics [5].

Diuretic Sites Along the Nephron

The diuretics commonly used in children can be grouped into five classes, based on the sites of action along the nephron (Fig. 1) (Table 1). Most diuretics circulate in blood bound to albumin and are secreted by proximal tubule transporters into the tubular fluid, where they can inhibit their apical targets. Spironolactone and tolvaptan access their receptor targets from the basolateral surface of tubular cells. Only mannitol is freely filtered through the glomerulus.

Proximal Tubule

The convoluted and straight portions of the renal proximal tubule are richly supplied with mitochondria and are designed for bulk reabsorption of nearly 60% of the filtered load of salt and water

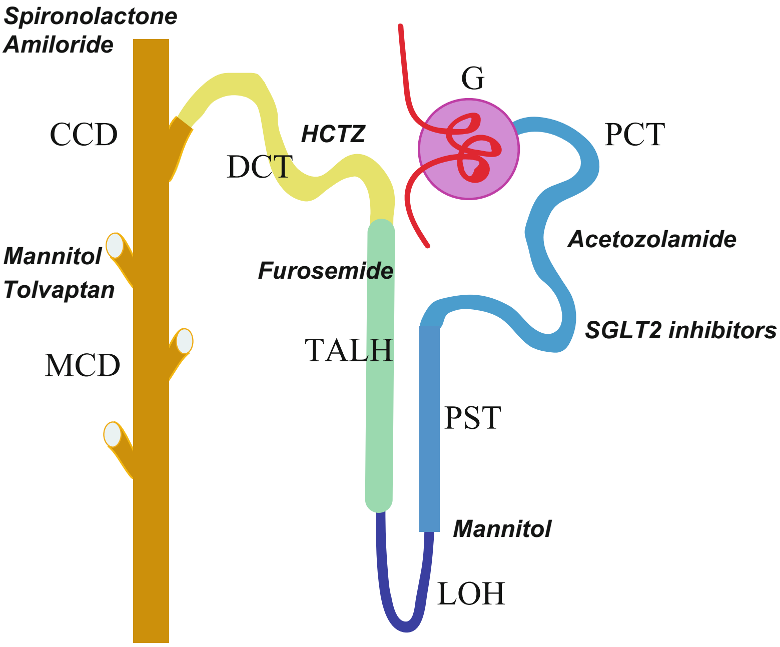

Fig. 1 Diuretic sites of action along the nephron Acetazolamide and gliflozins are transported from blood into the proximal convoluted tubule (PCT segments S1 and S2) lumen, where they inhibit bicarbonate and glucose transport, respectively. Mannitol is freely filtered by the glomerulus and causes an osmotic diuresis by opposing water flux through aquaporin-containing cells in the proximal tubule (AQP1), thin descending limb of the Loop of Henle (AQP1), and collecting ducts (AQP2). Also secreted into the proximal tubule, loop diuretics (e.g., furosemide) inhibit the apical $NaKCl_2$ transporter in the thick ascending limb of the loop of Henle (TAL) while thiazide diuretics (e.g., hydrochlorothiazide) inhibits the apical NaCl symporter in the early portion of the distal convoluted tubule. Cells of the late distal convoluted tubule, connecting tubule, cortical and medullary collecting ducts express the apical, electrogenic sodium channel (ENaC) which is inhibited directly by amiloride (secreted into tubular fluid) and indirectly by spironolactone (in blood). Tolvaptan (in blood) blocks the basolateral vasopressin receptor (V2R) in cortical and medullary collecting ducts, causing a water diuresis

and 95% of organic solutes. Thus, any drugs or pathologic processes that interfere with proximal tubular reabsorption have the potential to cause massive diuresis – overwhelming compensatory distal reabsorption mechanisms. **Acetazolamide,** the first such proximal diuretic, was discovered in 1941. It acts at the apical surface of cells in the proximal tubule as a noncompetitive inhibitor of carbonic anhydrase, thereby decreasing H+ availability for the Na+/H+ exchanger at the luminal membrane (Fig. 2). This limits proximal tubule sodium reabsorption and impairs H+ secretion into the lumen, trapping bicarbonate there. Early studies by Leaf et al. demonstrated its diuretic properties in patients with congestive heart failure, but disappointingly, they found that continued use of acetazolamide inevitably led to acidosis and relative resistance to its diuretic effects after a few days [6]. Although these properties limit the use of acetazolamide as a first line diuretic, it has found several unexpected applications in acute glaucoma (reducing formation of aqueous humor in the ciliary body), high altitude pulmonary edema (mechanism unclear), raised intracranial pressure (reduced production of cerebrospinal fluid), and correction of severe alkalosis [7].

In recent years, a new class of drugs (**gliflozins**) has been developed that inhibit the apical sodium-glucose reabsorption transporter (SGLT2) in the S1 and S2 segments of the renal proximal tubule. Empaglifozin, canagliflozin, and dapagliflozin have been studied extensively for the treatment of Type 2 diabetes mellitus in adults. Despite compensatory mechanisms along the renal tubule, all members of the SGLT2 inhibitor family are

Table 1 Diuretic action in the nephron segments

Diuretic	Target	Nephron Segment	Use	Dose/kg	MAX	Comment	References
Acetazolamide	CAII	PT	Papilledema	8–30 mg/kg q8h		Accumulates in ESRD	[82]
			Glaucoma	3–6 mg/kg q8h	1000 mg/day	3–10 mg/kg <12 years	[83]
			Alkalosis	5 mg/kg q24h X 3 days			
			High altitude	2.5 mg/kg q12h		Day before and of ascent	[84]
			Seizure disorder	1–4 mg/kg q6 h	16 mg/kg/day		[85]
Canagliflozin	SGLT2	PT	Diabetic nephropathy	100–300 q24h			[86]
Empagliflozin	SGLT2			10–25 mg q24h			[87]
Dapagliflozin	SGLT2			5–10 mg q24h			[88]
Mannitol	Aquaporin channels	PT, TDL CCT,MCT	Cerebral edema	0.5–1.0 g/kg (20–30 min)	Serum osm < 320		[89]
			Dialysis disequilibrium	0.5–1.0 g/kg	50% during hr. 1, 25% during hrs 2–3		[90]
Furosemide	NA-K-2Cl (NKCC2)	TAL	Starting intermittent oral	1.0–2.0 mg/kg	100 mg/day	Not dialyzable	[91]
			Starting intermittent iv	0.5–2.0 mg/kg			
			Titrate to achieve diuresis	1–2 mg/kg q6-8hrs	6 mg/kg/day	Can add HCTZ or amiloride	[92]
			Continuous infusion				
			Loading dose	0.1 mg/kg			
			Infusion rate	0.05–0.4 mg/kg/hr			[93]

			Neonate (po) <31 weeks GA	0.5–2.0 mg/kg q24h			[94]
			Neonate (po) >31 weeks GA	0.5–2.0 mg/kg q12h			
			Neonate (iv) <31 weeks GA	1 mg/kg q24h			
			Neonate (iv) >31 weeks GA	1 mg/kg q12-24 h			
Bumetanide	Na-K-2Cl (NKCC2)	TAL	Edema	0.01–0.1 mg/kg/ dose		2.5% of furosemide dose	[95]
			Continuous infusion	6ug/kg/hr			[46]
Hydrochlorothiazide	NaCl symporter (NCC)	DCT1	Edema, furosemide resistance	0.25–1.0 mg/kg q12h		With furosemide or amiloride	[97]
			Calcium nephrolithiasis	0.25–1.0 mg/kg q12h			
Chlorthalidone	NaCl symporter (NCC)	DCT1	Edema or hypertension	0.1–0.3 mg/kg q24h	2 mg/kg/day	Monotherapy or with furosemide	[97, 98]
Metolazone	NaCl symporter (NCC)	DCT1	Edema	0.1–0.2 mg/kg q12h		With furosemide	[99]
Amiloride	Na + channel (ENaC)	DCT2, CCD, MCD	Hypertension	0.4–0.625 mg/ kg/day		With HCTZ or furosemide	[17]
			Edema	0.625 mg/kg/day		Monitor K+	[17]
			NDI	0.1 mg/kg q8h	12 mg/m^2/day	With HCTZ 2 mg/kg/d	[100]
Spironolactone	Na + channel (ENaC)	DCT2, CCD, MCD	Edema	1–3 mg/kg/day		With furosemide (ratio 2:5)	[92]
			Ascites	1–3 mg/kg/day	6 mg/kg/day	With furosemide or alone	[66]
Tolvaptan	ADH receptor II (V2R)	CCD, MCD	Hyponatremia	0.05–0.3 mg/kg/ day		Start at low dose, titrate	[101]

CAII – cytoplasmic carbonic anhydrase, PT – proximal tubule, SGLT-2 – sodium glucose transporter, TDL – thin descending limb, CCT – cortical collecting duct, medullary collecting duct, TAL – thick ascending limb, HCTZ – hydrochlorothiazide, NKCC2 – Na/K/Cl2 , DCT – distal collecting duct, ENaC – electrogenic apical sodium channel, ADH – anti-diuretic hormone, V2R – vasopressin 2 receptor

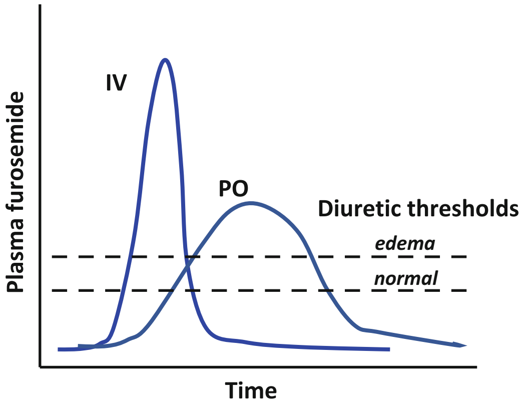

Fig. 2 Tubular fluid threshold for loop diuretics Intravenous furosemide circulates in blood >95% bound to albumin, largely precluding filtration through the renal glomerulus (Ellison CJASN 2019). However, it is rapidly transported across the basolateral membrane of proximal tubular cells into tubular fluid at concentrations that inhibit the TAL apical $NaKCl_2$ transporter; elimination half-life is also rapid, and duration of diuresis is about 2 hours. When administered orally, furosemide is absorbed slowly, reducing the serum peak concentration but extending the period of time during which tubular fluid concentrations exceed the threshold for transporter inhibition; diuresis lasts for about 6 hours. However, in edematous states, the threshold for $NaKCL_2$ inhibition is elevated and significantly higher doses are required to achieve the same diuresis. Similarly, proximal tubular cell injury may limit the rate of delivery into tubular fluid, increasing the minimal effective dose to 2–4 mg/kg

moderately potent diuretics. In rats with type 2 diabetes mellitus, Masuda et al showed that oral gliflozins cause a sustained increase in urine volume (80%), natriuresis (40%), and free water clearance (15%) [8]. Although gliflozins do not block the SGLT1 sodium-glucose transporter in the proximal tubule S3 segment, this secondary high-affinity low-capacity transporter is overwhelmed when the upstream mechanism is lost. There is now great interest in the cardiorenal protective effects of these drugs, in addition to their ability to improve diabetic control.

Thick Ascending Limb of the Loop of Henle

Furosemide is an organic anion that blocks the apical Na/K/Cl2 transporter (NKCC2, encoded by SLC12A1) (Fig. 3) and inhibits by interacting with chloride-binding site – without being transported into cell. Furosemide must gain access to the tubular fluid compartment in order to be effective. Bioavailability of an oral dose is about 50–60% [9]. Since furosemide circulates in blood bound to albumin, its clearance through the glomerulus is very low and enters the tubular fluid primarily by proximal tubule secretion pathway – using OAT1 and OAT2 transporters for uptake at the basolateral surface [9]. The drug is concentrated in the proximal tubular cell and diffuses passively across the apical surface into the proximal tubular lumen. Other loop diuretics such as bumetanide, torsemide, and neseritide have slightly better oral bioavailability (80%) and more rapid uptake from the intestine, but shorter half-life.

Furosemide and the other loop diuretics all target the same $NaKCl_2$ transporter, NKCC2, (encoded by the SLC12A1 gene) at the apical surface of cells in the thick ascending limb of the loop of Henle. At very high dose, these drugs inhibit up to 25% of the filtered load of sodium [10]. While TAL cells transfer sodium and chloride ions across the basolateral membrane into the interstitium, potassium is pumped back across the apical membrane via the ATP-dependent ROMK channel. This establishes a positive electrical potential in the lumen that enhances reabsorption of calcium and magnesium through paracellular pathways. By ablating lumen positivity, furosemide compromises divalent cation reabsorption, causing hypercalciuria, and hypermagnesuria. Since the TAL is impermeable to water, normal NKCC2 function dilutes tubular fluid and establishes a hyperosmotic medullary interstitium. Conversely, furosemide blunts the osmotic gradient that allows vasopressin-driven water reabsorption along the collecting duct; this can be useful in allowing free water excretion in treating some hyponatremic states. With sustained NaKCC2 blockade, the resultant volume contraction powerfully stimulates aldosterone-driven reabsorption of sodium in the collecting tubule in exchange for hydrogen ion and potassium. Hypokalemia and alkalosis may present undesirable effects which limit furosemide in patients with normal tubular function or may be useful

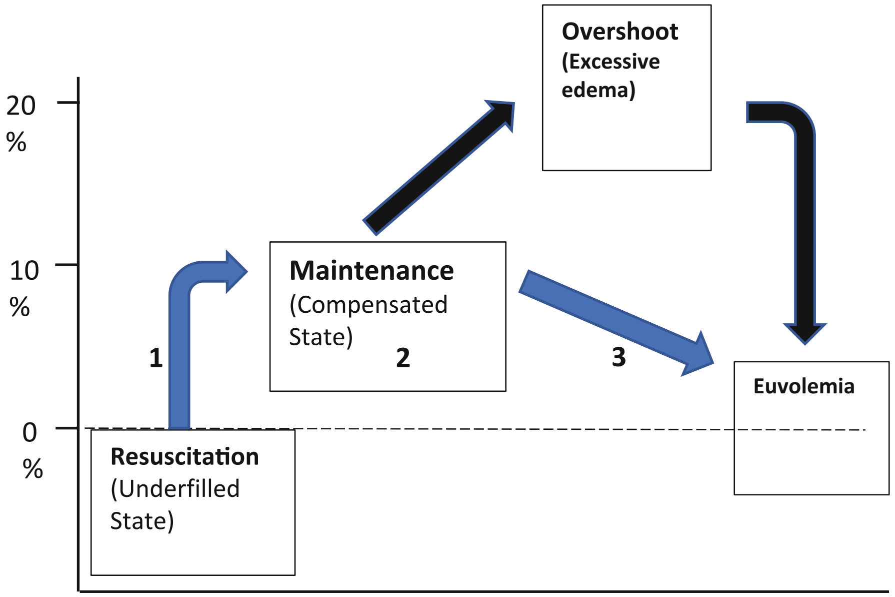
20 %
10 %
0 %
1
2
3
Resuscitation (Underfilled State)
Maintenance (Compensated State)
Overshoot (Excessive edema)
Euvolemia

Fig. 3 Fluid overload during treatment of shock
During resuscitation of critically ill children, intravenous fluid boluses are administered over minutes to hours in an effort to repair any dehydration and capillary leak that produce an "underfilled" state. As blood pressure and tissue perfusion are stabilized, attention turns to infusion of blood products, drugs, and intravenous alimentation. However, in the presence of acute kidney injury, oligoanuria may lead to fluid overload, organ edema, and organ dysfunction associated with poor outcome. Early diuretic use is essential to avoid excessive fluid overload beyond 10% of basal body weight. Sustained diuretic use accelerates return to euvolemia, and reduced hospital stay

consequences of loop diuretics in chronic or acute kidney failure.

Furosemide and the other loop diuretics can cause hearing loss in humans at very high doses >1 gram or infusion rates >250 mg/hr. [11]. The risk of hearing loss may be greater in patients with renal insufficiency (GFR <20 ml/min/1.73m^2) or concurrent ototoxic agents such as gentamicin [12]. Furosemide toxicity appears to involve inhibition of a $NaKCl_2$ homolog (encoded by the SLC12A2 gene) in the inner ear stria vascularis. Mice with targeted knockout of the SLC12A2 have structural damage in the inner ear and are deaf [13]. Ototoxicity seems to be linked to speed of the furosemide infusion and peak levels rather than total dose [14].

Early Distal Convoluted Tubule

Hydrochlorothazide and related thiazide-like diuretics (chlorthalidone and metolazone) are taken up into proximal tubular cells by the basolateral organic base secretory mechanism and secreted into the tubular lumen (Fig. 4). They target the downstream apical sodium chloride symporter (NCC, encoded by the SLC12A3 gene) in the early portion of the distal convoluted tubule (DCT1) [9]. NCC is an electroneutral transporter that can reabsorb 3–5% of the filtered sodium load, but, unlike the $NaKCl_2$ transporter in the TAL, it does not generate a negative luminal potential driving divalent cation reabsorption via the paracellular pathway. Instead, calcium and magnesium enter DCT1 cells via selective channels (TRPV5 and TRPV6, respectively) and are pumped across the basolateral membrane by diuretic-independent mechanisms [15]. On the other hand, the natriuretic effect of thiazide diuretics establishes a state of volume contraction which heightens $NaKCl_2$ transporter activity and indirectly enhances divalent cation reabsorption in the late TAL and DCT1 segments. In the DCT2 and collecting tubule, volume contraction drives

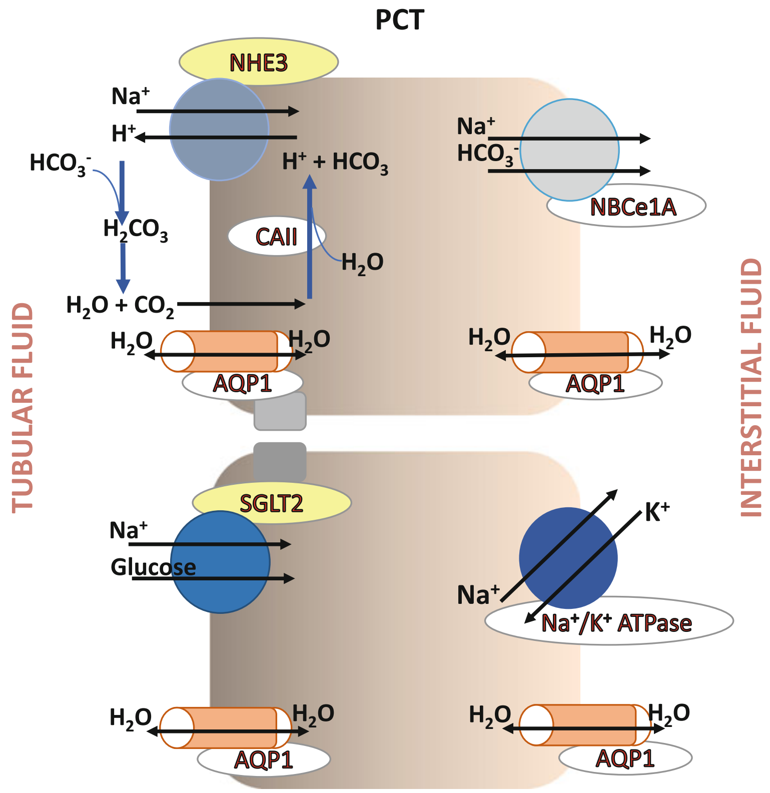

Fig. 4 Diuretics acting on the renal proximal tubule **Acetazolamide** is taken up by the organic anion transporters (OATs) into cells of the proximal tubules, where it inhibits the cytoplasmic (CAII) and brush border (CAIV) forms of carbonic anhydrase. By blocking the interconversion of carbonic acid and CO_2, the reabsorption of sodium bicarbonate from tubular fluid is inhibited, causing a moderate diuresis, alkalinization of the tubular fluid and proximal tubular acidosis. **Gliflozins** (e.g., empagliflozin, canagliflozin, and dapagliflozin) are taken up by basolateral organic anion transporters in the S1 and S2 segments of the proximal convoluted tubule and secreted into tubular fluid where they inhibit the apical Na/glucose transporter (SGLT2). The resultant glucosuria overwhelms the downstream low-capacity SGLT1 transporter, causing moderate water diuresis and mild natriuresis

K+ and H+ secretion as described below. Thus, thiazide diuretic inhibition of NCC in the DCT1 segment produces moderate natriuresis, hypocalciuria/hypomagnesuria, and hypokalemic alkalosis.

Late Distal Nephron (DCT2, CNT, CCD, MCD)

Amiloride is a pyrazine derivative that blocks the electrogenic apical sodium channel (ENaC) in principal cells throughout the late DCT2, connecting tubule (CNT), cortical (CCT), and medullary collecting tubules (MCT) [16]. ENaC is made up of four subunits, encoded by SCNN1A, SCNN1B, SCNN1G, and SCNN1D, respectively). While amiloride causes only a modest natriuresis (up to 1–2% of the filtered sodium load), it also blocks the ENaC-generated negative luminal charge that normally facilitates potassium (via the apical ROMK channel) and hydrogen ion secretion (via the hydrogen ion V-ATPase transporter) in nearby alpha-intercalated cells (Fig. 5). Thus, amiloride is often used in combination with thiazides or loop diuretics, to enhance diuresis while offsetting hypokalemia and alkalosis [17]. However, amiloride can only block a portion of tubular potassium loss; high urinary flow is now recognized to activate maxi-K channels in the alpha-intercalated cell apical membrane. This flow-dependent potassium secretion is

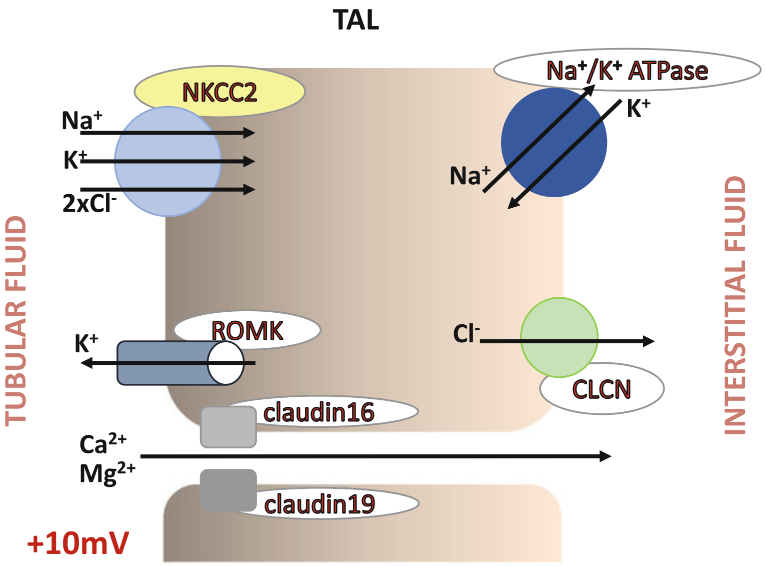

Fig. 5 Loop diuretics
Furosemide and other loop diuretics (e.g., bumetanide and torsemide) are secreted into proximal tubular fluid and target the apical $NaKCL_2$ transporter (NKCC2, encoded by SLC12A1) in the thick ascending limb of the loop of Henle (TAL). Normally, this electrically neutral transporter reabsorbs about 25–30% of filtered sodium. Sodium and chloride ions exit via the basolateral membrane while potassium is recycled to the lumen, generating a + 10 mV potential that drives calcium and magnesium reabsorption through the paracellular pathway. Furosemide blockade of NKCC2 rapidly causes natriuresis, hypercalciuria, hypermagnesuria; compensatory sodium reabsorption via the distal ENaC leads to hypokalemic alkalosis

independent of ENac and is unaffected by amiloride. By inhibiting ENaC function, amiloride is an effective antihypertensive agent in Liddle syndrome caused by autosomal dominant ENaC mutations that cause unregulated, hyper-reabsorption of sodium [18].

Interestingly, there are now reports that amiloride may be useful in treating anxiety disorders and has been adapted as a nasal spray for this purpose [19]. It is also useful in hereditary and lithium-induced nephrogenic diabetes insipidus in combination with thiazide diuretics. By causing volume contraction, water reabsorption is stimulated in the proximal tubule via aquaporin I channels.

Volume contraction produced by thiazides or loop diuretics predictably induces distal nephron compensatory mechanisms driven by increased blood levels of aldosterone. These effects include upregulation of principal cell ENaC itself, the basolateral Na-K ATPase, and the apical potassium secretory channel (ROMK). Thus, drugs that inhibit the aldosterone receptor, such as spironolactone and eplerenone, compromise ENaC function. Like amiloride, these drugs cause diuresis and block the generation of an electronegative luminal potential which drives potassium excretion and urinary acidification. They are useful as potassium-sparing diuretics in combination with thiazides or loop diuretics in heart failure and ascites. However, they must be used cautiously in patients with chronic kidney dysfunction, since they may cause unwanted hyperkalemia and acidosis. Significant side effects of spironolactone include gynecomastia in boys and vaginal bleeding in girls, but eplerenone lacks these complications.

Aquaporin-Containing Segments: Collecting Duct and Proximal Tubule/ Thin Descending Limb of the Loop of Henle.

Mannitol is an osmotically active polyalcohol sugar which is not orally absorbed and minimally metabolized. When injected intravenously, it is freely filtered through the glomerulus and is not reabsorbed during its passage along the renal tubule. Thus, it causes a water diuresis at nephron segments containing aquaporins, where water is freely permeable (Fig. 6). Its main site of action is in the proximal tubule and thin descending limb of the loop of Henle (AQP1) but also opposes vasopressin action AQP2 in the cortical and medullary collecting tubule (AQP2) [10]. Through a solvent drag effect, there is a mild natriuresis, but the water diuresis dominates and can eventually lead to hypernatremia. In normal individuals, about 80% of the drug is excreted in the urine over 10 hours; typical intravenous dose of 0.5–1.0 mg/kg infused over 2–6 hours usually produces urine flow rates of

Mannitol can be used to treat oliguria in states of fluid overload, but it is more commonly used to treat acute brain edema (1–2mg/kg over 30–60 minutes) through its effects on water movement out of the brain compartment and through indirect effects to contract the brain vascular volume [20]. It is also used to avert dialysis disequilibrium syndrome when pre-dialysis blood urea nitrogen exceeds 35 mM; a dose of 0.5–1.0 mg/kg is infused over the first 3 hours of a 5-hour dialysis session (50% in the first hour and 25% in each of the next 2 hours).

Mannitol diuresis protects against cisplatin-induced nephrotoxicity above the benefit from hyperhydration protocols alone [21]. Evidence in rats shows that cisplatin in the glomerular filtrate enters proximal tubular cells from the apical surface and contributes significantly to nephrotoxicity; mannitol diuresis markedly reduces cisplatin concentration in the tubular fluid [22].

Tolvaptan is a selective competitive inhibitor of the vasopressin receptor 2 and blocks aquaporin2-mediated water reabsorption in principal cells of the cortical and medullary collecting tubules (Fig. 6). By producing a pure water diuresis, it is useful in treating refractory hyponatremia in hospitalized adults with the syndrome of inappropriate antidiuretic hormone, congestive heart failure, or cirrhosis [23]. In hospitalized children, it has been used to treat severe hyponatremia of nephrotic syndrome, using a progressively increasing dose from 0.3–3.0 mg/kg/day to avoid overly-rapid correction of serum sodium or at a fixed dose of 0.35–0.45 mg/kg to prevent or correct mild hyponatremia following cardiac surgery [24, 25].

Clinical Use of Loop Diuretics in States of Acute Volume Overload

Figure 7 compares the basic pharmacokinetic patterns of iv and oral doses of furosemide in normal humans. Diuresis occurs when blood levels of drug generate sufficient intratubular concentrations to

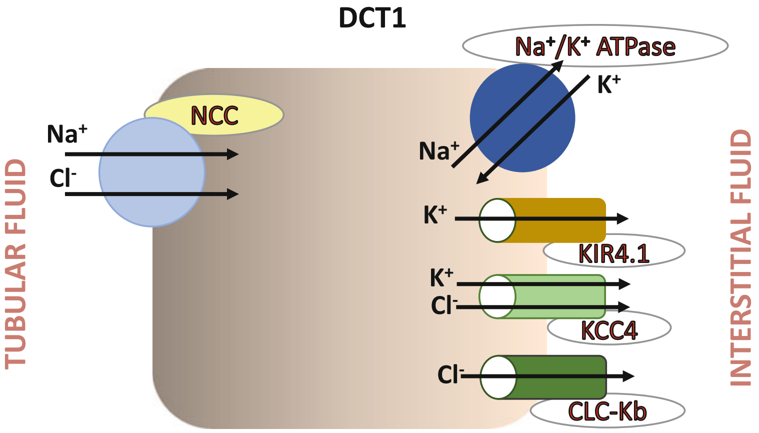

Fig. 6 Thiazide diuretics
Hydrochlorothiazide and other thiazide diuretics (e.g., metolazone, chlorthalidone) inhibit the apical electroneutral NaCl co-transporter (NCC, encoded by *SLC12A3*) in the early part of the distal convoluted tubule (DCT1) and can block up to 3–5% of the filtered load of sodium chloride. Like loop diuretics, the thiazides are secreted into the tubular lumen by proximal tubular to reach their apical DCT1 target

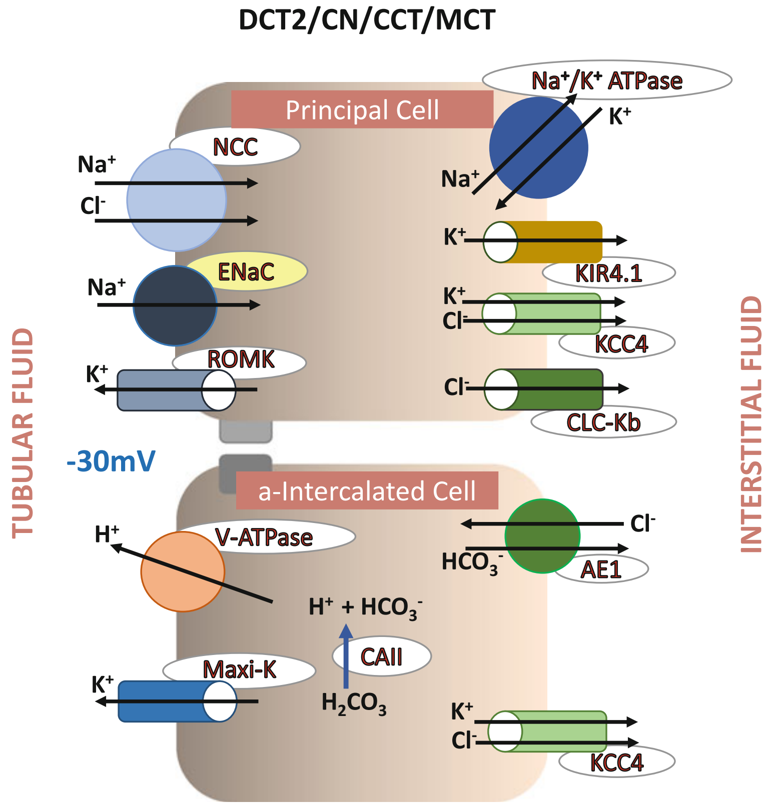

Fig. 7 Amiloride sensitive diuretics
Amiloride is a "potassium sparing" diuretic that inhibits the apical sodium channel (ENaC) expressed by principal cells throughout the late distal convoluted tubule (DCT2), connecting segment (CN), cortical (CCD), and medullary (MCD) collecting ducts. Amiloride secreted into tubular fluid can inhibit about 1–2% of the filtered load of sodium chloride. This electrogenic transport generates a –30 mV luminal potential that drives secretion of potassium via principal cell ROMK channels and both hydrogen (V-ATPase) and potassium (Maxi-K) ions from adjacent alpha-intercalated cells. Spironolactone and other "potassium sparing" diuretics (e.g., eplerenone and finerenone) exert a similar effect by inhibiting the mineralocorticoid receptor which regulates ENaC expression and function

exceed the threshold for significant $NaKCl_2$ transporter inhibition in the downstream thick ascending limb of the Loop of Henle. Note that the threshold to achieve diuresis is increased in many edematous states. Healthy adult volunteers taking a modest oral dose (0.25–0.4 mg/kg) of furosemide notice onset of diuresis within 2–4 hours and increased urine flow for about 6 hours [9]. After an intravenous bolus, onset of diuresis may be noted after 30–60 minutes. In either case, as diuresis subsides, the net natriuresis results in acute contraction of central volume that induces a period of increased sodium reabsorption, positive fluid balance, and kaliuresis, driven by distal nephron compensatory response. Thus, if a sustained state of relative volume contraction is desired, repeated doses of furosemide are needed – typically an oral dose q12 hours with oral potassium supplementation to prevent hypokalemia. Alternatively, a lower dose of loop diuretic may be combined with one of the potassium-sparing diuretics (e.g., spironolactone or amiloride) to minimize the amount of potassium supplement required.

With repeated administration of furosemide, there is often a progressive decrease in the diuretic response after a few days. This "braking" phenomenon reflects several compensatory mechanisms, including hypertrophy of the renal tubule (including the thick ascending limb of the loop of Henle) and increased aldosterone-driven stimulation of sodium reabsorption in the cortical collecting tubule. Sustained volume contraction may also compromise renal blood flow, uptake of furosemide via the proximal tubule OAT secretory system and thus the rate of furosemide delivery into the tubular lumen. The braking phenomenon has been well-described in adults and has been documented in children after about 72 hours of repeated dosing [26]. Kim et al. showed that positive fluid balance rises steadily from the third day of therapy onward, as the calculated natriuresis per mg of furosemide falls [26]. In the acute care setting, this effect is usually dealt with by increasing the furosemide dose, the frequency of furosemide doses or by the addition of distal acting diuretics. In the intensive care unit, a continuous infusion protocol may be considered. This may provide a more predictable diuresis with a modest reduction in total furosemide dose, but does not seem to produce a more potent diuresis than can be achieved with intermittent dosing [27].

Furosemide resistance can be seen in a variety of pathologic states. With fluid overload, bowel wall edema compromises absorption and the oral dose is estimated to be about twice the intravenous dose to achieve the same level of diuresis [9]. In chronic kidney disease, sodium reabsorption is suppressed in the residual nephrons as a compensatory response to falling GFR. Hypoalbuminemia causes an “underfilled” state which increases distal salt and water reabsorption that counters the upstream effect of furosemide on the TAL. There are no known mutations of the OAT that block furosemide secretion into the tubular lumen, but this becomes a critical issue in acute tubular necrosis or chronic progressive renal disease. In acute tubular necrosis, high doses of furosemide (4 mg/kg/dose) are needed to optimize the rate of delivery to the lumen and lift luminal furosemide levels above the threshold for $NaKCl_2$ inhibition. Since ATN also decreases renal clearance of furosemide, dosing frequency can be lengthened to every 24 hours in end-stage renal disease. Secretion of furosemide may be mildly diminished by NSAIDS which reduce renal blood flow or by drugs that share the OAT secretory mechanism, causing furosemide resistance by competing for delivery into the tubular lumen.

All loop diuretics target the apical $NaKCl_2$ transporter, but some may have pharmacokinetic advantages in certain clinical settings. Bumetanide is 40 times (mg/mg) more potent than furosemide and is approved by the FDA for children. Since bumetanide is cleared significantly by the liver, this ratio drops to about 20:1 in chronic renal failure; drug accumulation to ototoxic levels may be less likely with bumetanide than with furosemide but this has not been directly proven at equivalent diuretic doses. Small studies suggest intermittent bumetanide dosing from 0.015 to 0.1 mg/kg/dose every 6 to 24 hours for infants and children. There is no reported additional clinical benefit in infants with doses above 0.05 mg/kg and no increased diuresis compared to a 40X dose of furosemide. In adolescents, typical doses range from 0.5 to 1 mg/dose every 6 to 24 hours (max dose 10 mg/day).

Torsemide is two times more potent than furosemide and has a longer half-life. In adults with congestive heart failure, torsemide is associated with better functional improvement and decreased cardiac mortality [28]. However, there is no FDA-labeled indication for children and no strong rationale for torsemide continuous infusion. Ethacrynic acid does not contain a sulfonamide group, so is suitable for patients with sulfa allergies. It is 30% less potent than furosemide and has greater incidence of ototoxicity. The FDA has approved ethacrynic acid for treating edema in children older than 1 year old at a dose of 1 mg/kg/dose every 8 to 12 h (max 50 mg/dose) but its use has decreased because of its greater irreversible ototoxicity compared to furosemide [29].

Diuretics in Specific Clinical Settings

Acute Post-Infectious Glomerulonephritis (APSGN)

In APSGN, glomerular filtration may fall dramatically but tubular function is relatively well preserved. Rapid onset of fluid overload is accompanied by hypertension and hyperkalemia which respond readily to diuretics. At presentation, early introduction of furosemide may avert the need for hospitalization by reducing the volume-dependent component of hypertension and whole-body potassium accumulation. Unlike acute tubular necrosis where proximal tubule secretion of diuretics is sharply impaired, an oral dose of furosemide (2 mg/kg) is efficiently transferred to the tubular lumen and causes timely diuresis and kaliuresis over 6–8 hours. Intermittent oral dosing over the next days is often sufficient for outpatient management.

Nephrotic Syndrome

In steroid-sensitive nephrotic syndrome, edema may be massive but is likely to resolve after 2 weeks of prednisone therapy. Furthermore, severe hypoalbuminemia causes marked contraction of central volume and overly aggressive diuresis may cause hypotension. Thus, edema is usually managed with low salt intake alone without diuretics. However, some children with massive edema may experience skin breakdown, respiratory difficulty, gastrointestinal symptoms, or mechanical discomfort. They may also develop significant hyponatremia. In these settings, cautious diuresis typically involves oral furosemide (1–2 mg/kg/day) allows partial mobilization of edema and improvement in hyponatremia. When response to furosemide is suboptimal, iv furosemide can be administered toward the end of a 2–4 hour infusion of 25% albumin (0.5 g/kg) [30]. However, albumin infusion moves interstitial fluid into the vascular compartment and enhances furosemide-induced diuresis but, if given too rapidly, may rarely precipitate pulmonary edema [30]. To reverse this shift of fluid into the alveolar space, vasodilators (to increase intravascular capacitance) such as nifedipine or nitroglycerin patch are said to be rapidly effective but have not been carefully evaluated.

Meena et al have reported an alternative protocol in which oral tolvaptan (0.5–1.0 mg/kg/day) administered in combination with intravenous furosemide (3–4 mg/kg/day) for 2 days [31]. This approach avoids the rare pulmonary complications of albumin infusion and uses a diuretic which is unaffected by the serum albumin concentration. However, prospective studies in larger numbers of children are needed to refine this protocol and establish safety.

Chronic Congestive Heart Failure

Diuresis is essential in the setting of congestive heart failure. A typical situation is the baby who has undergone cardiac surgery for a congenital defect and is left with reduced ejection fraction despite ionotropic agents and an ACE inhibitor to reduce afterload. Peripheral and pulmonary edema may initially respond to a loop diuretic (e.g., 0.5 mg/kg furosemide twice daily), but may become complicated by emergence of the "braking phenomenon" and rise in serum creatinine.

The first dilemma is whether to increase furosemide dose or increase the frequency of furosemide doses. Since primary myocardial failure may represent an underfilled state, pediatricians may be reluctant to increase diuresis because further volume reduction could reduce renal perfusion and lower GFR. However, it must be remembered that congestive heart failure causes widespread venous congestion; the rise in intraperitoneal venous pressure and the increase in renal edema alter the pressure gradient driving filtration across the glomerular capillary wall and reduces GFR. Diuresis is crucial to reduce edema in this setting and warrants an increase in furosemide dosing [28]. Since the diuretic threshold for loop diuretics is elevated in diuretic states, it is common practice to double the furosemide dose (or number of doses per day) to achieve a significant increase in diuresis [10]. Alternatively, diuresis can be accomplished by adding a distal-acting diuretic (the choice of which is guided by serum potassium). A thiazide is typically added if serum potassium is normal [32], whereas a potassium sparing diuretic (aldactone or amiloride) may be preferable if there is hypokalemia [33]. To avoid compromise of cardiac filling pressures, diuresis should usually be limited to 1–2% of body weight per day.

Although ACE inhibitors are useful for afterload reduction in heart failure, it may be useful to hold (or reduce) these drugs during periods of cardiac decompensation. Since successful diuresis is dependent on GFR, continuation of ACE inhibitors may compromise intraglomerular pressure, causing elevated serum creatinine and further resistance to furosemide.

Treatment of Shock in the PICU

The principles of resuscitation during critical illness are based on improving oxygen delivery relative to oxygen demand. Therapeutic interventions are predicated on improving gas exchange in the lungs, optimizing cardiac output, and normalizing systemic blood pressure. Pulmonary gas exchange may be compromised by fluid overload while cardiac output is dependent on preload, myocardial contractility, and afterload. Thus, manipulation of total body water and its distribution is fundamental to critical care. The goal is to achieve a s*ufficient volume*, vasomotor tone, and cardiac contractile performance that sustain tissue perfusion relative to metabolic needs. While aggressive volume expansion is crucial during the early phase of circulatory failure, this inevitably leads to total body water overload, organ edema, and dysfunction and a requirement for aggressive use of diuretics during the recovery phase.

A recent systematic review (44 studies with 7507 children) addressed the negative impact of acute fluid overload on worsening of respiratory function (pulmonary edema), development of acute kidney injury, longer intensive care stay, and death [34]. The odds of death were increased 6% for every 1% increase in fluid overload, independent of disease severity. Fluid overload may affect all critical organs [35]:

- Cerebral edema: impaired cognition, delirium.
- Myocardial edema: conduction disturbance, impaired contractility, diastolic dysfunction.
- Pulmonary edema: impaired gas exchange, reduced compliance, increased work of breathing.
- Renal interstitial edema: reduced renal blood flow, increased interstitial pressure, reduced glomerular filtration rate, uremia, salt and water retention.
- Hepatic congestion: impaired synthetic function, cholestasis.
- Gut edema: malabsorption, ileus.
- Tissue edema: poor wound healing, wound infection, pressure ulceration.

Vincent and De Backer [36] proposed that treatment of shock could be envisioned in distinct temporal phases.

1. **Rescue (minutes; severe shock):** administration of lifesaving fluid to achieve perfusion pressure and cardiac output compatible with immediate survival (shock correction). Fluid therapy is given as rapid boluses.
2. **Optimization (hours; unstable patients):** organ rescue by maintaining tissue perfusion. This needs careful monitoring of cardiac output, mixed venous oxygen saturation (SvO2), and lactate levels. Fluid challenges may be used to assess reversibility of oliguria.
3. **Stabilization (days; stable patient):** maintenance of stable volume expansion to preserve organ function, while avoiding excessive fluid overload. Aim for zero or negative fluid.
4. **De-escalation (days to weeks; recovery):** organ recovery with the goal of mobilizing accumulated fluid providing treatments to help achieve negative fluid balance.

Goldstein [37] later simplified this scheme, allowing focus on diuretics:

A) Resuscitation Phase, combining immediate and subsequent steps in volume expansion – diuretics are not usually employed.

B) Maintenance Phase, as the underlying cause of shock is dealt and attention turns to nutrition, blood products, and secondary complications (diuretics are important to maintain neutral fluid balance).

C) Recovery Phase (diuretics are important to achieve reduction of fluid overload) (Fig. 3).

Diuretic therapy is often introduced during transition from the resuscitation to maintenance phases. Loop diuretics are the mainstay of this effort, due to their rapid onset action and potency. In stable patients, intermittent doses of furosemide range from 0.5 to 2 mg/kg/dose every 6 to 12 h (maximum 6 mg/kg/dose or 200 mg/dose in adults). Naïve patients might need a smaller initial dose. While loop diuretics are useful in critically ill children with residual renal function, they are ineffective when GFR is fallen to zero or proximal tubule cell death precludes delivery of drug into the tubular lumen. Renal dimercaptosuccinic acid scans may be useful in detecting viable proximal tubular cells, so that recovery from acute tubular necrosis may be possible [38]. In the setting of oligo-anuria following resuscitation from shock, a challenge with a single high-dose (2–4 mg/kg) of furosemide may unmask residual GFR.

While diuretics do not prevent or reverse ischemic kidney injury [39], they are essential for managing fluid overload during the maintenance phase of therapy for shock. Vasudevan has suggested that the net volume expansion needed to compensate for “third-spacing,” blood pressure support and delivery of critical medications is ideally kept below 10% of pre-morbid weight [40]. However, the need for non-resuscitation fluid during the maintenance phase (blood products, intravenous medications, parenteral nutrition, and fluid boluses to maintain blood pressure in unstable patients) frequently leads to excessive fluid overload (Fig. 8) and secondary organ edema. Barhight et al. found in a study of 14,483 critically ill children, that about 75%, 20%, and 5% received non-resuscitation fluids accounting for $<10\%$, 10–20%, and $> 20\%$ fluid overload, respectively, by the third day of admission [41]. Every 1% fluid overload was associated with a 1% increase in mortality, and a 1% decrease in the likelihood of a ventilator-free day [41]. This suggests that a continuous estimate of fluid load as a percent of pre-morbid body weight should be kept (when this can be obtained from parents or estimated from recent medical records) and that aggressive diuretic use should be used in the maintenance phase to avoid fluid overload above 10% of baseline body weight.

In patients who exhibit resistance to loop diuretics, thiazides (acting in the distal convoluted tubule) and/or diuretics acting in the collecting tubules (e.g., amiloride or spironolactone) are frequently added. The latter potassium-sparing agents are preferable in patients with hypokalemia. However, a recent retrospective study of 448 patients showed that spironolactone supplementation did not significantly reduce the need for potassium supplementation in one cardiac pediatric intensive care unit [42].

In patients with hemodynamic instability, loop diuretics may be administered as a continuous infusion. This appears to have less effect on central venous pressure, oxygen saturation, and heart rate compared to intermittent dosing [43]. In 2014, Miller et al published an extensive literature review on the use of continuous-infusion (CI) loop diuretics in critically ill children

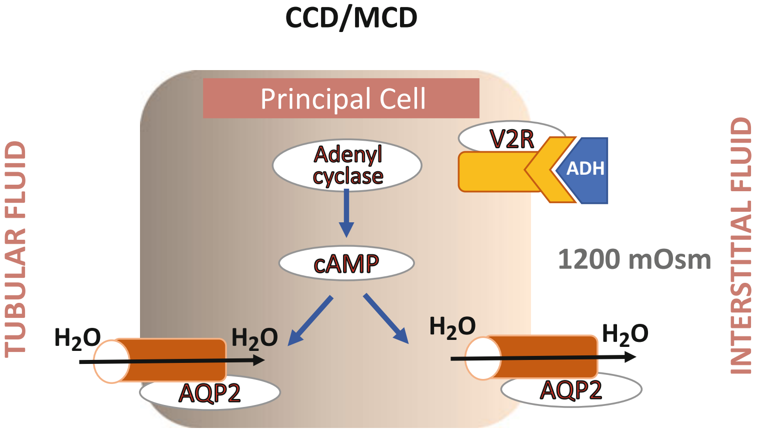

Fig. 8 Osmotic diuretics
Mannitol is a polyalcohol that is freely filtered at the glomerulus and is not reabsorbed by the renal tubule. Through its osmotic properties, mannitol opposes flux of water in aquaporin-containing cells of the medullary collecting ducts and thin Loop of Henle. In the collecting ducts, water reabsorption through the aquaporins can be blocked directly by **Tolvaptan**

[44]. Compared with intermittent dosing, there were no significant differences in urine output, but continuous infusions appear to produce a more-controlled diuresis, resulting in less variability in hemodynamic parameters. In general, a loading dose ranged from 0.1 to 2.4 mg/kg was used, followed by a continuous infusion ranging from 0.05 to 0.2 mg/kg/hour (maximum 0.4 mg/kg/hour). Pharmacokinetic evaluations and clinical evidence indicate that a higher furosemide dose of 0.2 mg/kg/hour may be required for patients with decreased renal function. It is important to note that all of the patients included these studies were below 4 years old.

Bukley et al. [45] studied bumetanide infusion doses in 40 neonates and children (1.6 ± 2.6 years). The reported mean initial dose was 30 mcg/kg/h (range: 1 to 100 mcg/kg/hour) and the mean maximum dose was 50 mcg/kg/hour (range: 3 to 200 mcg/kg/hour); a complete safety analysis was lacking in this trial. In 2015, McCallister et al. [46] suggested a dose from 1 to 10 mcg/kg/h based on their retrospective study of 95 patients (median 0.2 years, range 0 to 15.7 years). Negative fluid balance was achieved in 76% of patients within 48 hours of initiation; of note, 60% of patients were receiving concomitant intermittent diuretics.

Miller et al. [47] reported the effect of continuous ethacrynic acid infusion at a median initial dose of 0.13 mg/kg/h and a maximum dose of 0.17 mg/kg/h in a retrospective pilot study in nine patients. Ricci et al. [48] conducted a clinical trial of 36 pediatric post-operative cardiac surgery patients (mean age: 135 ± 181 days), reporting a mean effective dose of 0.22 ± 0.13 mg/kg/hour. These authors recommended an initial rate is 0.1 mg/kg/hour, to be titrated to a maximum reported infusion rate of 0.5 mg/kg/hour. There have been no published studies on the use of torsemide continuous infusion in children.

Congenital Heart Surgery

Fluid overload is common following congenital heart surgery, due to a combination of fluid resuscitation (intra- and postoperatively), hemodynamic instability, repair of blood loss on the day of surgery, postoperative acute kidney injury (around 50%), and systemic inflammation after cardiopulmonary bypass [49]. Fluid overload in the early postoperative period is associated with delayed sternal closure, longer ICU stay, prolonged inotrope and ventilation needs, and higher mortality and morbidity [50]. Post-cardiac surgery cases with an open sternum require aggressive volume removal to permit sternal closure. A recent systematic review and meta-analysis suggests that postoperative fluid overload is significantly linked to elevated risk of prolonged hospital stay, acute kidney injury, and mortality in pediatric patients undergoing cardiac surgery [51]. The main treatment for these patients is diuretic therapy. Furosemide is typically introduced when fluid overload exceeds 10% of basal body weight. However, recent studies suggest that fluid overload as low as 5% may be associated with poorer clinical outcomes [51]. There is increasing interest in low dose (3 mg/kg) aminophylline [52] and fenoldopam [53] as they seem to improve diuresis in combination with furosemide and reduce urinary acute kidney injury biomarkers. Onder et al observed in a retrospective chart review (200 patients) that the intraoperative use of aminophylline was more effective than furosemide in reversal of oliguria in the early postoperative period. The aminophylline group also required less renal replacement therapy [54]. However, a prospective randomized trial done by Axelrod et al in 2015 found no effect of aminophylline to prevent acute kidney injury in children recovering from cardiac surgery performed with cardiopulmonary bypass [55].

A common complication of protracted furosemide following cardiac surgery is the development of a contraction alkalosis which is thought to delay weaning from the respirator in some cases. Lopez et al recently showed that addition of a 2-day oral course of acetazolamide augmented diuresis and reduced serum bicarbonate and pCO_2 [56].

Acute Lung Injury

Both ARDS and acute lung injury (ALI) present with an increase in pulmonary vascular permeability due to regional endothelial damage [57]. Several studies show association between fluid overload and worse respiratory outcomes [58] [59]. In the Fluid and Catheter Treatment Trial (FACTT) [60] published in 2006, adults with ALI were randomized to a conservative versus liberal fluid management. The conservative strategy improved lung function and decreased the duration of mechanical ventilation without increasing non-pulmonary organ failure. Valentine et al. [59] demonstrated that increased fluid balance at day 3 in children with ALI was independently associated with fewer ventilator-free days but not with mortality. Silversides et al. conducted a systematic review and meta-analysis of this issue and found that patients with ARDS, sepsis, or SIRS (both adults and children) managed with early reduction of volume overload had increased ventilator-free days and decreased ICU stay compared with standard care; the effect on mortality was uncertain. Larger trials will be needed to optimize fluid strategies for managing acute pulmonary injury in children [61].

Traumatic Brain Injury/Cerebral Edema

Traumatic brain injury (TBI) might elicit a different response to fluid overload than the acutely injured lung, as recently described by Stulce et al [62]. This retrospective chart review of 138 patients with severe TBI demonstrated fluid overload (>10%) on day 10, but this did not contribute to mortality, longer ventilation time, increased risk of acute kidney injury, or increased stay in intensive care. In the absence of lung injury, fluid balance in severe traumatic brain injury relies on two mechanisms: first, the state of blood-brain barrier function and extracellular fluid kinetics [63] and, second, the severity of neurogenic pulmonary edema [64]. Thus, the risks of fluid overload may be more complex in isolated TBI [65].

Ascites

Independent of its cause, diuretics have a role in mobilizing moderately severe ascitic fluid which cannot be managed by salt restriction alone. Because of high aldosterone levels in patients with ascites, Bavdekar suggests oral furosemide (0.5–1.0 mg/kg twice daily) combined with spironolactone (1–3 mg/kg daily) to achieve net negative fluid balance of about 10 ml/kg/day. Spironolactone should be increased gradually every 3–5 days to reach this target, but more aggressive diuresis tends to compromise GFR [66]. Sabri recommends starting with spironolactone alone (2–3 mg/kg daily for 4–5 days, then doubling the dose if needed) [67]. This approach takes note of the long half-life of spironolactone; onset of diuretic effect may be delayed for several days and peaks at 2 weeks. Sabri reserves furosemide (0.5–1.0 mg/kg) for those with suboptimal response [67].

Neonates

Premature (<30 weeks gestation) newborns commonly develop bronchopulmonary dysplasia, with a requirement for oxygen during periods of mild fluid overload; nearly 40% of such newborns are exposed to diuretics to optimize pulmonary function [68]. Case reports in adults suggest that very high doses of furosemide (>1 gram/day) may be associated with hearing loss. In 2018, a large multicenter study was conducted of 1220 premature newborns (gestational age = 23–29 weeks) with exposure to standard doses of furosemide (about 1–2 mg/kg/day) for more than 28 days to test for an effect of furosemide exposure on hearing loss [68]. After accounting for a wide range of other possible risk factors (including comorbidity severity), some degree of hearing loss was noted in 14% of babies exposed to furosemide, compared to 10% among those who never received furosemide. However, this difference was not statistically significant.

Chemotherapy Protocols

In many standard chemotherapy protocols, hyperhydration (twice maintenance rate of intravenous fluid) is commonly used to induce high urinary flow rates to minimize direct toxicity of chemotherapeutic agents (e.g., cisplatin). However, hypoalbuminemia, kidney infiltration by leukemic cells, AKI, or tumor lysis syndrome from high tumor burden may compromise renal excretion of the fluid load. In this setting, intermittent loop diuretic (e.g., intravenous furosemide 0.5–1.0 mg/kg every 6 hours) is commonly used to target a urinary flow rate of >0.5 ml/kg/hr. In the setting of acute kidney injury, higher doses of furosemide can be given at increased dosing intervals (e.g., 2–4 mg/kg every 12 to 24 hours).

Acetazolamide can be used to hasten and or enhance urinary alkalinization during chemotherapy. During a period of intravenous sodium bicarbonate shortage in North America, several groups of adult oncologists compared various protocols for alkalinization of the urine during high dose methotrexate treatment [69]. They found that the time to alkalinization (15 hours) using a standard $NaHCO_3$ infusion of 250 meq $NaHCO_3/m^2$/day) could be achieved with an infusion containing less $NaHCO_3$, when oral acetazolamide was added to inhibit proximal tubular carbonic anhydrase and spill some of the filtered load of bicarbonate into the distal nephron. They reasoned that the latter approach might not be sustainable for 3 days (to allow complete methotrexate excretion) but that additional oral $NaHCO_3$ supplementation might suffice at that stage.

Adapting these observations to children, a practical protocol for timely alkalinization of the urine ($pH > 8.0$) prior to chemotherapy is as follows:

Infusion of 0.45% NaCl containing 75 meq/L $NaHCO_3$ at twice maintenance rates (3 L/m^2/day) plus acetazolamide (200 mg/m^2) at the outset. Intravenously, acetazolamide acts within minutes and alkalinizes the urine for 4–5 hours; if given orally, acetazolamide acts within 60–90 minutes and lasts 8–12 hours. By lowering the proximal tubular threshold for bicarbonate, addition of acetazolamide establishes early urine $pH > 8.0$ (to prevent obstructive methotrexate crystallization in distal tubules) and allows timely chemotherapy.

Kidney Transplantation

At the time of kidney transplantation, particularly in smaller recipients, large fluid volumes are administered to expand the central venous volume prior to unclamping and assure blood pressures sufficient to perfuse the adult allograft. In the first post-op day, additional positive fluid balance may be needed to compensate for "third-spacing." At 48 hours, most recipients have been fluid overloaded by 10–20% of preoperative body weight. Gradual repair of volume load is then usually managed by allowing the renal allograft to excrete the fluid load without full replacement. Whenever possible, diuretics are avoided since over-diuresis might risk sub-optimal renal perfusion. However, in the setting of delayed graft function or excessive fluid overload, a diuretic may be needed. Since the fresh renal allograft appears to be unusually responsive to loop diuretic, most transplant physicians use furosemide, but start at greatly reduced doses (e.g., 0.05–0.1 mg/kg/dose).

Prolongation of Urine Output in End-Stage Renal Disease

High dose loop diuretics have been used in oliguric dialysis patients to increase urine output, diminish the need for aggressive fluid removal during hemodialysis or peritoneal dialysis sessions. In a North American study of oliguric adult patients on peritoneal dialysis, Bargman et al reported that for each 250 ml of urine/day, the relative risk of death was decreased by 36% [70]. Lamarche et al studied 11 oliguric adults on peritoneal dialysis and found that a single oral dose of furosemide (500 mg) increased mean urine volume from 420 ml to 800 ml [71]. The International Paediatric Peritoneal Dialysis study examined residual renal function in children during the first year of peritoneal dialysis and noted an 80% decrease in the risk of developing anuria among those who were treated with a loop diuretic [72]. Shen et al. studied decline of renal function in the first year of peritoneal dialysis in 66 children and noticed a modest delay

(2 months) in the loss of urine output among those on diuretics [73].

Nonrenal Applications of Acetazolamide

Aside from its effects on the renal proximal tubule, acetazolamide is used in the management of glaucoma to reduce intraocular pressure. Carbonic anhydrase in the ciliary body of the eye generates the bicarbonate-rich fluid which is transported into the anterior chamber. Thus, acetazolamide reduces intraocular pressure by inhibiting aqueous humor production [50]. Interestingly, fluid shifts during hemodialysis may induce acute angle closure glaucoma in susceptible individuals [74], making it reasonable to consider single pre-dialysis doses of acetazolamide in this setting. However, studies in children for this unusual application are lacking. In the brain, acetazolamide reduces excessive intracranial pressure by blocking carbonic anhydrase in the choroid plexus, reducing production of cerebrospinal fluid [74]. In papilledema complicating growth hormone therapy, doses of 10–25 mg/kg/day, divided 3–4 times doses of 15–25 mg/kg/day (divided three or four times daily) typically reduce intracranial pressure over days to weeks and may be sustained for months [75]. Care must be taken to correct acidosis and avoid over-diuresis during this period.

Diuretic Use in Calcium Nephrolithiasis

HCTZ acts on DCT to block NaCl cotransporter, subsequent volume contraction induces increased sat reabsorption on TAL, hyperpolarizing electropositive lumen and driving calcium reabsorption through paracellular channels to produce hypocalciuria. A similar effect causes hypocalciuria in Gitelman syndrome vs Bartter syndrome where TAL reabsorption of salt is compromised, causing hypercalciuria. These effects provide the rationale for the use of HCTZ in individuals with hypercalciuria and kidney stones. Hydrochlorothiazide consistently reduces urinary calcium in children [76]. Liern reported that the relative risk of recurrent stone formation (3.3), episodes of hematuria (2.5), and osteopenia (3.0) is increased among those with hypercalciuria. Interestingly, thiazides reduce the risk of recurrent nephrolithiasis even when urinary calcium is within the normal pediatric [77] or adult ranges [78]. However, long-term success with this strategy is undercut by poor adherence. Although hydrochlorothiazide (0.5 mg/kg twice daily) reliably decreases urinary calcium, long-term nonadherence is reported in one-third of patients and hypercalciuria recurs in 44% [79].

SGLT2 Inhibitors in Diabetic Nephropathy

The gliflozins exert both cardioprotective and renoprotective effects in patients with diabetic nephropathy and it now appears that these benefits may extend to nondiabetic forms of heart and kidney disease as well. By inhibiting sodium/glucose reabsorption in the proximal convoluted tubule, increased solute is delivered to the macula densa, inducing constriction of the afferent arteriole, and dropping intraglomerular pressure [80]. A hemodynamic effect that reduces GFR slightly in the first weeks is followed by attenuation of progressive renal insufficiency that becomes evident by the end of the first year [80]. Cardiorenal protection is thought to occur, in part, by the reduction of central volume achieved through sustained diuresis and reduction in blood sugar; systolic blood pressure typically falls by 3–5 mmHg. Most clinical trials indicate that the renoprotective effects are additive to those of ACE inhibitors [81]. However, in some patients, concurrent diuretics may have to be reduced or curtailed to avoid excessive volume contraction during gliflozin therapy.

At present the gliflozins have not been approved for individuals <18 years old. However, since the early stages of diabetic nephropathy begin in childhood, there will inevitably be interest in use of these drugs in young people. Expertise from pediatric nephrologists (about the diuretic effects) and from pediatric endocrinologists (about effects on glycemic control and ketoacidosis) will be essential in learning how to apply these drugs safely in children.

Dosing and Pharmacokinetic Properties of Commonly Used Diuretics in Pediatrics (Table 2)

Table 2 Diuretic pharmacokinetics

Diuretic	Onset	Peak	Duration	Half-life	ESRD	Reference
Acetazolamide po	1–1.5 hours	2–4 hrs	18–24 hrs	2.4–5.8	Long T1/2	[31]
Acetazolamide iv						
Canagliflozin po	<24 hrs	1–2 hrs	24 hrs	11 hrs	Adjust, eGFR<45	–
Empagliflozin po	<24 hrs	1.5 hrs	24 hrs	12.4 hrs	Adjust, eGFR <45.	–
Dapaglifozin po	<24 hrs	2.0 hrs	24 hrs	12.9 hrs	Long T1/2	–
Mannitol iv	Diuresis 1–3 hrs			1.5–2.5 hrs	T1/2 = 6–36 hrs	[31]
	ICP 15-30 min		1.6–6 hrs			
Furosemide po	30-60 min	1–2 hrs	6–8 hrs	0.5–2.0 hrs	T1/2 = 9 hrs	[9]
Furosemide iv	15 min	0.5 hrs	2 hrs	0.5–2 hrs	T1/2 = 9 hrs	
Bumetanide po	0.5–1.0 hours	1–2 hrs	4–6 hours	1 hr	50% hepatic	
Bumetanide iv	2–3 min	15–30 min	2–3 hours	6–15 hrs (neonates)		[94]
				2.5 hrs <2mo		
				1.5 hrs 2-6mo		
				1.-1.5 hrs adults		
Hydrochlorothiazide po	2 hrs	4–6 hrs	6–12 hrs	6–15 hrs	Long T1/2	[96]
Chlorthalidone po	2.6 hrs	2–6 hrs	24–48 hrs	14–20 hrs	Long T1/2	[98]
Metolazone po	1 hr		>24 hrs	8–15 hrs	Long T1/2	[99]
Amiloride po	2 hrs	6–10 hrs	24 hrs	6–9 hrs	Long T1/2	[17]
Spironolactone po		2.6–4.3 hrs	2–3 days	1–2 hrs	Hepatic	[92]
Tolvaptan po	2–4 hrs	4–8 hrs	<24 hrs	3 hrs	Long T1/2	[101]

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Part VII

Cystic Kidney Diseases and Related Disorders

Autosomal Dominant Polycystic Kidney Disease

49

Melissa A. Cadnapaphornchai and Djalila Mekahli

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Abstract

Pierre Rayer first described the cystic degeneration of the kidneys in 1841. Later Felix Lejars formally coined the term "polycystic kidney disease" in 1888. However, to date, autosomal dominant polycystic disease (ADPKD) is still a disease without cure despite representing the most common monogenic cause of renal failure. Research accelerated after *PKD1* and *PKD2* were identified in 1994 and 1996, several underlying mechanisms that cause ADPKD have been elucidated, and major advancements in diagnosing, prognosticating, and understanding the natural course of the disease resulted in the approval of the first likely disease-modifying treatment in vasopressin V_2 receptor antagonists.

Awareness is growing that the clinical course of ADPKD begins in childhood and the wind of change in the management of more nephron-protective strategies and lifestyle modifications with early referral and follow-up are evolving. This chapter aims to deeply describe the genetic background, pathophysiology, clinical manifestations, and main therapeutic options including the future perspective. The currently accepted views on the optimal management in childhood ADPKD with regard to ethical and practical aspects will be summarized.

Keywords

Autosomal dominant · Polycystic kidney disease · Children · Hypertension

Synonyms

ADPKD; Cystic kidney disease; Polycystic kidney disease

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease, affecting 1 in 1000 individuals of all races and ethnicities and accounting for nearly 5% of end-stage kidney disease (ESKD) [121, 152]. The cardinal manifestation of ADPKD is bilateral progressive cystic dilation of renal tubules with eventual compression of normal renal parenchyma, kidney enlargement, and loss of renal function, with approximately half of affected individuals reaching ESKD by 60 years of age. It is a systemic disease, with extrarenal manifestations including hepatic, splenic, and pancreatic cysts, vascular dysfunction and early onset hypertension, and intracranial aneurysms. Although historically considered a disease of adults, it has become clear in recent decades that ADPKD has important clinical manifestations in childhood and in some cases as early as in utero [36]. Early identification of high risk patients is critical to allow timely intervention to alter long-term prognosis. The purpose of this chapter is to review the genetics, pathophysiology, clinical manifestations, diagnostic considerations, and potential therapies associated with pediatric ADPKD.

Genetics, Structure, and Function of Mutated Proteins

ADPKD is a heterogenetic disorder most often caused by mutations in the *PKD1* gene located on chromosome 16p13.3 encoding polycystin-1 (PC1) (~78% of cases) or in the *PKD2* gene located on chromosome 4q22 encoding polycystin-2 (PC2) (~15% of cases) [117]. Many years were spent looking for a "third locus" with recent studies identifying ADPKD due to mutations in *GANAB* (~0.3%), *DNAJB11* (~0.1%), and ALG9 ([114, 32, 7]).

PKD1 is a very large gene, spanning 53 kb of genomic DNA with 46 exons including a 14.5 kb transcript (International Polycystic Kidney Disease Consortium [113]). A region of the 5′ end of *PKD1* from exons 1 to 33 is duplicated six times approximately 15 Mb proximal to the *PKD1* gene on chromosome 16. The high sequence homology of these six pseudogenes (P1-P6) to *PKD1*, nearly 100% in exonic sequence, complicates genetic sequencing and requires locus-specific long-range PCR products to specifically amplify *PKD1* [118]. The gene encodes the large novel 4304–amino acid 460 kD protein product polycystin-1 (PC1) whose precise function is unknown. PC1 is an integral membrane protein with a large extracellular N-terminal domain (~3000 amino acids), 11 transmembrane domains, and a C-terminal cytosolic domain of 200–225 amino acids. The extracellular domain of PC1 contains a physiologically important G-protein-coupled receptor proteolytic site. The cytoplasmic tail of PC1 has a coiled-coil motif and two nuclear loop localization sequences. In addition to the GPS cleavage site, cleavage of PC1 at the C-terminal tail releases a soluble 35 kD fragment [26]. The carboxy terminal domain of PC1 undergoes proteolytic cleavage, and nuclear translocation of the PC1 carboxy-terminus plays a role in regulating gene expression. These features suggest that PC1 is a large, multifunctional molecule involved in carbohydrate motif recognition, ligand binding, and Ca^{2+} regulation. It may engage in cell-cell and/or cell-matrix interactions which regulate signal transduction pathways mediated by cell surface protein–protein interactions or directly participate in regulating transcriptional programs through the cleaved cytoplasmic tail. PC1 is expressed in multiple tissues including kidney, liver, pancreas, intestine, and cerebral blood vessels, all sites of pathologic changes in ADPKD, as well as in the lung, testes, and other tissues. More than 1250 pathogenic *PKD1* variants have been identified, spread throughout the gene, of which up to 70% are unique to a single family. The *TSC2* and *PKD1* genes lie adjacent to each other in a tail-to-tail orientation on chromosome 16 at 16p.13.3. Deletions inactivating both genes lead to the *TSC2/PKD1* contiguous gene syndrome which is associated with early onset (usually in infancy) severe cystic kidney disease with features of tuberous sclerosis and high risk for rapid loss of kidney function in childhood and early adulthood [120].

PKD2 has been linked to chromosome 4q13-q23 [107]. It expresses a 5.4 kb mRNA which encodes a 968–amino acid polypeptide, polycystin-2 (PC2) [94]. PC2, also called TRPP2, is a calcium–permeable nonselective cation channel [61]. PC1 and PC2 are thought to interact through their C-terminal tails, and the resulting complex has been proposed to regulate intracellular calcium influx [57]. Of more than 250 known *PKD2* pathogenic variants, spread throughout the gene, half are unique.

Mutations in *PKD1* and *PKD2* produce similar phenotypes. However, the natural course of ADPKD is significantly influenced by the causative gene. Specifically, *PKD1* mutations are associated with larger kidneys with more cysts as compared to *PKD2*; however, the rates of cyst growth do not differ, suggesting that PKD1 is more severe because cysts develop earlier and not because they grow faster [3, 63]. Mutations in *PKD1* are thus not surprisingly associated with earlier onset of ESKD (58 vs. 79 years) than with *PKD2* mutation [30]. Moreover, among patients with *PKD1* mutations, ESKD is reached earlier with truncating mutations vs non-truncating mutations (55.6 vs. 67.9 years).

Recently mutations in other genes including *GANAB*, *DNAJB11*, and *ALG9* have been associated with ADPKD. *GANAB,* which localizes to chromosome 11q12.3 with genomic size 21.9 kb, has been proposed to account for ~0.3% of ADPKD and encodes the catalytic subunit of glucosidase II (GIIα). It was identified as a candidate gene by whole exome sequencing in several families with ADPKD whose routine genetic screening was not informative [114]. A unique feature in many of these families was the lack of significant kidney enlargement or dysfunction despite cystic kidney disease. Furthermore, there was a high prevalence of hepatic cysts and polycystic liver disease (PLD). Through combined genetic human and cellular studies, Porath et al. demonstrated that mutations in *GANAB* caused ADPKD and autosomal dominant polycystic liver disease (ADPLD) and that loss or reduction of GIIα is associated with maturation and localization defects in PC1 and PC2.

Mutations in *DNAJB11*, located at chromosome 3q27.3, have also been shown to cause atypical ADPKD [32] with non-enlarged kidneys with multiple small cysts and significant interstitial fibrosis, with evolution to renal insufficiency after the sixth decade. *DNAJB11* encodes one of the main cofactors of the endoplasmic reticulum chaperone BiP, a heat-shock protein required for efficient protein folding and trafficking. It has been proposed that *DNAJB11*-related disease induces a hybrid phenotype with features of ADPKD from PC1 maturation defects and autosomal dominant tubulointerstitial kidney disease due to intra-cytoplasmic retention of tubular proteins like uromodulin.

In a cohort of genetically unresolved polycystic kidney and liver disease, Besse et al. [7] identified *ALG9* mutations as another rare source of atypical ADPKD. *ALG9* encodes an endoplasmic reticulum enzyme which builds N-glycans, and inactivation is associated with impaired PC1 maturation. Nearly 90% of carriers of *ALG9* mutations over age 50 years have multiple kidney cysts. However, the spectrum of cystic renal and liver involvement is variable. More comprehensive studies are needed to fully characterize the natural course of these rare forms of ADPKD.

Finally, it is important to consider the impact of combined and hypomorphic genetic variants in the ADPKD phenotype. Although fully penetrant biallelic pathogenic variants in *PKD1* or *PKD2* are predicted to be incompatible with life, biallelic hypomorphic *PKD1* mutations or the combination of a classical and an ultra-low penetrant PKD1 mutation can cause severe early onset ADPKD [39, 104, 118, 145]. Digenic disease with variants in *PKD1* and *GANAB* has also been identified in a child with early onset ADPKD [146]. Severe ADPKD may also occur with combined transmission of mutations in *PKD1* or *2* and other cystic nephropathy genes such as *HNF1β* or *PKHD1* [5, 6].

Pathophysiology of Kidney Cyst Formation

ADPKD is characterized by kidney cysts that gradually form and increase in number and size. Kidney cysts may arise from any segment of the nephron, most typically the distal nephron and the collecting duct, but originate from only about 1% to 5% of nephrons in total. Unlike autosomal recessive polycystic kidney disease (ARPKD), in which the cystic dilatations are fusiform in nature and remain in connection with the tubular lumen, in ADPKD the enlarging cysts ultimately detach from the tubular lumen and urinary space. This eventually leads to marked enlargement of the kidneys with vascular remodeling and interstitial fibrosis in advanced ADPKD. Although every cell in the kidneys of ADPKD patients carries the same monoallelic genetic germline mutation, only about 100 cysts per kidney are formed in a lifetime. Several hypotheses are suggested to explain this phenomenon [64, 68] (Fig. 1). It has been suggested that an additional somatic mutation in the second wild-type allele, as a recessive cellular mechanism, leads to cyst formation later in life, that is, the "second hit hypothesis." This is supported by a recent next-generation sequencing study that demonstrates somatic *PKD1* and *PKD2* mutations in the epithelium of 90% of cysts measuring over 1.5 cm [134, 156]. More recently, the importance of the dosage of functional PC protein below a critical threshold (20–30% of normal values) has been described as the "threshold hypothesis." This hypothesis is influenced by the type of mutation(s) and local stochastic factors [69, 81, 109]. In addition, the "third hit hypothesis" proposes the role of inflammation, ischemia-reperfusion, nephrotoxicity, and crystal deposition as additional triggers to induce cyst growth [62, 133, 137, 151]. Finally, the "snowball effect" suggests that local and mechanical effects of the cyst can disrupt flow in the adjacent tubule segments as well as in blood and lymphatic vessels [83]. Indeed, cysts that developed from the collecting duct in inner medulla have the potential to destroy the function of hundreds – if not thousands – of upstream nephrons that ordinarily drain

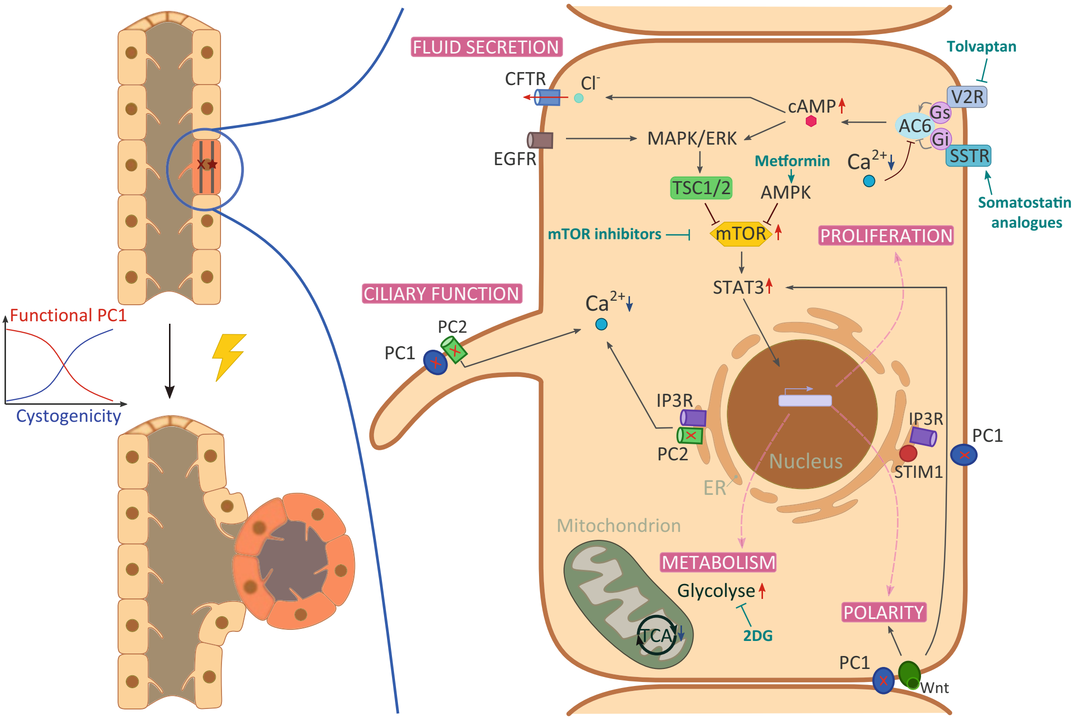
Functional PC1
Cystogenicity
FLUID SECRETION
CFTR
Cl-
cAMP
EGFR
MAPK/ERK
Metformin
TSC1/2
AMPK
mTOR inhibitors
mTOR
PROLIFERATION
Tolvaptan
V2R
Gs
AC6
Gi
SSTR
Ca2+
Somatostatin analogues
CILIARY FUNCTION
Ca2+
STAT3
PC2
PC1
IP3R
PC2
Nucleus
ER
IP3R
STIM1
PC1
Mitochondrion
METABOLISM
Glycolyse
2DG
TCA
POLARITY
PC1
Wnt

Fig. 1 Molecular mechanisms leading to cystogenesis in ADPKD. Left: The transformation of a tubular cell to a cystic cell requires a somatic mutation ("second hit," marked with an asterisk) to complement the germline mutation (marked with x), the decline of functional PC1 levels ("threshold hypothesis" represented by the graph), and/or renal stress factors ("third hit" represented by the lightning). Right: Several cellular alterations are required to generate cystic cells (marked in pink boxes). As the polycystins are expressed in the cilia, altered ciliary function including reduced ciliary Ca^{2+} signaling is proposed to trigger cyst formation. PC1 and PC2 also affect Ca^{2+} signaling by regulating IP$_3$R and STIM1. Reduced intracellular Ca^{2+} levels will also lead to enhanced cAMP (through less suppression of AC6), which in turn triggers enhanced Cl$^-$-driven fluid secretion and enhanced mTOR activity. Altered mTOR and Wnt signaling can trigger enhanced proliferation by stimulating STAT3-mediated transcription. Altered Wnt signaling will also influence cell polarity. Finally, ADPKD cells are also characterized by metabolic reprogramming involving enhanced glycolysis and reduced TCA. Several possible chemical modulators that are under investigation as drugs to suppress ADPKD progression are shown in the pathways they target. 2DG: 2-deoxy-D-glucose; AC6: adenylate cyclase 6; AMPK: 5′ adenosine monophosphate-activated protein kinase; cAMP: 3′,5′-cyclic adenosine monophosphate; CFTR: Cystic fibrosis transmembrane conductance regulator; EGFR: epidermal growth factor receptor; Gi: Gi protein alpha subunit; Gs: Gs alpha subunit; IP3R: Inositol 1,4,5-trisphosphate receptor; MAPK/ERK: mitogen-activated protein kinase/extracellular signal-regulated kinase; mTOR: mechanistic target of rapamycin; PC1: polycystin 1; PC2: polycystin 2; SSTR: Somatostatin receptor; STAT3: Signal transducer and activator of transcription 3; STIM1: Stromal interaction molecule 1; TCA: tricarboxylic acid cycle; TSC1/2: tuberous sclerosis 1/2; V2R: Vasopressin receptor 2; Wnt: Wingless/Int-1 Wnt pathway

into the tubule arcade served by the cystic collecting duct [58].

The mechanism(s) of cystogenesis in ADPKD are complex and not fully elucidated (Fig. 1). Loss-of-function of the polycystins modifies the regulation of multiple signaling pathways, including intracellular Ca^{2+} homeostasis, mechanistic target of rapamycin (mTOR) activation, and 3′-5′-cyclic adenosine monophosphate (cAMP) levels, ultimately resulting in abnormal ciliary structure or function, increased cell proliferation, altered apoptosis and autophagy, enhanced fluid secretion, abnormal cell-cell/matrix interactions, and alterations in cell polarity. PC1 and PC2 can both be found in the primary cilium, which is an antenna-like structure present on most types of mammalian cells, and therefore ADPKD is considered a ciliopathy. Initially the PC complex was thought to function as a sensor of laminar flow, impacting intracellular Ca^{2+}-flux upon stimulation [37, 88]. However, this hypothesis has recently been questioned and remains controversial. While reduced ciliary Ca^{2+} influx after mechanical stimulation has long been postulated as one of the primary events in cystogenesis, recent evidence shows little Ca^{2+} influx into cilia under physiological conditions, and ciliary Ca^{2+} concentration does not change upon mechanical bending of primary cilia in multiple cell types.

It has been further suggested that the abnormal Ca^{2+} signaling in ADPKD could result from altered PC2 function as an ER Ca^{2+}-release channel. Indeed, at the ER, PC2 is thought to regulate intracellular Ca^{2+} release through interactions with the inositol 1,4,5-trisphosphate receptor (IP_3R), the ryanodine receptor, and stromal interaction molecule-1 (STIM1). PC2 enhances Ca^{2+} release from the ER by stimulating the activity of the IP_3R, whereas PC1 inhibits this process by reducing PC2-IP_3R interactions via a mechanism involving STIM1 and the PI3K/AKT pathway [19, 93]. At the plasma membrane, PC1 regulates cell adhesion and cell matrix composition via regulation of planar cell polarity through wingless/integrated (Wnt) signaling, increased fluid secretion in cysts driven by the cystic fibrosis transmembrane conductance regulator (CFTR), and activation of proliferative pathways affecting intracellular signaling, for example, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK), signal transducer and activator of transcription 3 (STAT3), and mTOR. An additional hallmark of ADPKD is the alteration of cell proliferation of cystic kidney epithelial cells. Mutations in the PKD genes have been observed to increase mTOR activity by the inhibition of the TSC1/TSC2 complex via a mechanism that includes the cAMP-dependent B-Raf/ERK pathway [143]. Recently, aberrant aerobic glycolysis has been observed in PC1-deficient epithelial cells which triggers a metabolic reprogramming of the cell similar to cancer cells (the "Warburg effect"). In vitro characterization of the metabolomic profile of PC1-deficient mouse tissue shows an important metabolic shift with increased glycolysis, glutaminolysis, and reduced tricarboxylic acid cycle (TCA). In vivo analysis of 13C-glucose tracer in murine models of PKD has highlighted an increased glucose uptake and lactate production with increasingly severe disease manifestation. Furthermore, the administration of the non-metabolizable glucose analogue 2-deoxy-D-glucose (2DG) slows down disease progression in *PKD1* −/− cells [103, 112, 119]. Although these metabolic alterations seem to play a central role for the disease progression, the mechanisms behind it remain unknown.

Structural Kidney Disease: Implications for Symptoms and Disease Progression

The clinical phenotype of ADPKD can vary widely, especially in childhood. We know however that progressive structural kidney disease occurs from early in life and far in advance of any signs or symptoms. Recent studies have therefore highlighted the importance of total kidney volume (TKV) as an early and important marker of disease progression in ADPKD prior to any detectable change in glomerular filtration rate (GFR).

Cohort studies in children at-risk for ADPKD (one parent known to have ADPKD) demonstrated that despite normal kidney function, affected children have larger kidneys which grow faster than those of unaffected children and that affected children with hypertension have larger kidneys than those with normal blood pressure [15, 41, 43]. Studies utilizing MRI in ADPKD adults described a direct correlation between TKV and hypertension or proteinuria and an inverse correlation between TKV and estimated GFR (eGFR) [23, 60, 102]. In affected adults, height-corrected TKV over 600 mL/m predicts functional decline to chronic kidney disease stage 3 within 8 years [24].

Serial MRI has confirmed the progressive increase in TKV and total cyst volume (TCV) in children with ADPKD [16]. Higher TKV, TCV, and fractional cyst volume (the percent of TKV occupied by cysts), as well as increase in cyst volume over time have been observed in hypertensive as compared to normotensive children with ADPKD. The annual increase in TKV observed over a five-year study period was 7.4% per year overall and 8.1% per year in young adult subjects (18–21 years of age). This growth rate was higher than that observed in ADPKD adults (5.3% per year, [60]) and may reflect the impact of fibrosis in later stage disease. Thus the value of increasing TKV as an early marker of disease progression, particularly in childhood, cannot be understated.

Renal Manifestations

Hypertension. With advances in renal replacement therapy, cardiovascular disease is now the leading cause of death in ADPKD. Onset of hypertension prior to 35 years of age is a concerning prognostic factor in adults with ADPKD and has been associated with faster progression to ESKD compared to patients with later onset hypertension [48]. Hypertension occurs with increased frequency in children with ADPKD, with an overall prevalence of 20–40%, significantly higher than in the general pediatric population, and increasing in prevalence with age [41, 90, 91, 125]. Of ADPKD children with normal blood pressure followed at a tertiary care center for a five-year period, 52% of children with baseline blood pressure in the 75th to the 95th percentile and 28% of children with blood pressure below the 75th percentile but with 10 or more kidney cysts developed systemic hypertension [15].

These observations are worrisome, as studies in the general population support tracking of blood pressure from childhood to adulthood and suggest that elevated blood pressure in childhood may initiate chronic alterations in the cardiovascular system such as increased left ventricular mass index and premature atherosclerosis which increase long-term risk of cardiovascular morbidity and mortality [4, 28]. Moreover, studies in both ADPKD children and adults demonstrate a consistent positive correlation between blood pressure and kidney size. This relationship is particularly strong in children and is consistent across both normal and elevated systolic and diastolic blood pressures [14]. Of particular concern, increased TKV and fractional cyst volume associated with hypertension in ADPKD children does not appear to be impacted by degree of blood pressure control nor use of angiotensin converting enzyme inhibition (ACEI) and is associated with decreasing eGFR over time, albeit within the normal range [15, 16].

24-hour ambulatory blood pressure monitoring (ABPM) is an important management tool in pediatric ADPKD. ABPM is more valid and reliable than casual blood pressure measurement [129], and ABPM values are closely associated with left ventricular hypertrophy in children [10] and with kidney disease progression or death in adults with chronic kidney disease [1]. Isolated nocturnal hypertension with normal daytime blood pressure has been reported in 16–18% of children with ADPKD [91, 125]. Such children would not otherwise be identified without the use of ABPM, and early detection of hypertension is critical to mitigate long-term morbidity and mortality.

The pathophysiologic mechanisms of hypertension in pediatric ADPKD are poorly understood, and it is often assumed that the pathogenesis is similar in children and adults (reviewed in [13]). It has been proposed that cyst expansion leads to compression of the renal vasculature with local ischemia/hypoxia and intrarenal activation of the renin-angiotensin-aldosterone system (RAAS) which then mediates cyst growth, fibrosis, and hypertension [21, 123]. In support of this theory, adult ADPKD patients were shown to have increased plasma renin activity and serum aldosterone concentrations in both the supine and upright positions in response to ACEI treatment as compared to matched controls with essential hypertension, with a significant decrease in renal vascular resistance in the ADPKD group after ACEI treatment [21]. Histologic studies have also localized angiotensinogen, renin, ACE, angiotensin II, and the AT1 receptor within cysts and dilated tubules in ADPKD kidneys [85]. However, a direct link between RAAS and kidney cyst growth has yet to be demonstrated. Other proposed mechanisms of hypertension in this condition include activation of the sympathetic nervous system [20, 53], which is also known to stimulate RAAS, and decreased nitric oxide production.

Kidney dysfunction. The association between impaired urinary concentrating capacity and extent of structural kidney disease in ADPKD has long been recognized in both adults [46] and children [41] but there are little data to support higher prevalence of polyuria, urinary frequency, or nocturnal enuresis in ADPKD children. While microalbuminuria has been demonstrated to be an important marker of progressive disease in adults [22], this has not been found in repeated cohorts in pediatric ADPKD [15, 17]. However, overt non-orthostatic proteinuria is suggestive of advanced disease in childhood. Declining GFR is a late feature of the disease attributed to glomerular hyperfiltration in remnant nephrons. Once adults with ADPKD manifest falling eGFR, a progressive decline of 4–6 mL/min per year is anticipated [75]. Children with ADPKD demonstrate glomerular hyperfiltration when compared to healthy controls [154]. When present, this finding is associated with faster decline in kidney function and higher rate of kidney enlargement over time [65]. Fortunately however the vast majority of children with ADPKD maintain kidney function within the "normal" range given these considerations.

Abdominal/flank pain. Approximately 10–20% of children with ADPKD complain of abdominal or flank pain [49], a rate which is comparable to the general pediatric population [79, 136]. Potential etiologies in ADPKD include cyst expansion particularly when associated with capsular irritation, cyst hemorrhage, cyst infection (often associated with fever), urinary tract infection (UTI), or urolithiasis. Severe chronic pain can also occur in the setting of significant kidney enlargement and may be associated with chronic early satiety and/or reflux symptoms. Tumors associated with ADPKD in childhood are exceedingly uncommon. Pain due to cyst rupture typically resolves within 2–7 days without needing intervention other than rest, analgesics, and adequate hydration. Management of chronic pain due to ADPKD should include a multidisciplinary pain team approach to support self-management skills and to avoid chronic narcotic use. Nonsteroidal anti-inflammatory drugs are often avoided in ADPKD due to potential impact on renal function.

Macroscopic hematuria. Macroscopic hematuria can occur in the setting of cyst hemorrhage, urolithiasis, or UTI. Passage of clots can be associated with urinary tract pain but this scenario is uncommon with cyst hemorrhage. Such gross hematuria is typically self-limited. Macroscopic hematuria has been associated with enlarged TKV in adult ADPKD ([47], [73]), and its occurrence prior to 35 years of age portends worse kidney survival in affected adults [31]. However, there is no evidence currently for a predictive association between macroscopic hematuria and long-term kidney outlook in childhood ADPKD [41, 127]. This issue requires further study.

Other urinary tract concerns. As in adults, studies have suggested an increased frequency of urinary tract infection in children with ADPKD [41]. The prevalence of UTI may be overestimated however as culture results are not always reported. In any case, children with ADPKD do not seem to be at particular risk for complicated UTI, thus evaluation and treatment should proceed per routine practice. Cyst infection can rarely occur in childhood, often in association with fever, ill appearance, increased inflammatory markers, and sterile pyuria.

Nephrolithiasis occurs in 20–35% of adults with ADPKD but appears significantly less common in children with ADPKD. Evaluation and treatment should proceed as per routine for children with nephrolithiasis.

Extrarenal Manifestations

Cardiac disease and vascular dysfunction. Children with ADPKD have increased left ventricular mass index (LVMI) as compared to healthy children even with equivalent blood pressure [160]. There is also good correlation between both systolic and diastolic blood pressure and LVMI in children with ADPKD [14]. Not surprisingly, hypertensive ADPKD children have higher LVMI than affected children with normal blood pressure [71]. However, children with ADPKD and blood pressure in the 75th to the 95th percentile also demonstrate an increase in LVMI which is comparable to that of hypertensive ADPKD children and is significantly higher than that of affected children with lower blood pressure [14]. These findings suggest that significant cardiovascular manifestations are already occurring early in children with ADPKD and even within normal range of blood pressure.

Cardiac valvular abnormalities have also been reported to occur with increased frequency in ADPKD. For example, mitral valve prolapse was described in 12% of children with ADPKD, as compared to 3% of healthy siblings [71]. More recent experience however suggests lower prevalence than historically described. Routine echocardiogram looking specifically for cardiac valve disease is recommended only in the setting of concerning cardiac exam.

ADPKD is also associated with chronic vascular dysfunction as assessed by flow-mediated dilation (FMD) and by pulse wave velocity (PWV) of large elastic (aorta and carotid) arteries [8, 78, 149]. Both of these measures are independent predictors of future cardiovascular events and mortality. A recent study in children and young adults with normal eGFR demonstrated that vascular dysfunction is an early finding in ADPKD, with affected participants demonstrating significant impairment in FMD and increase in PWV compared to healthy controls [98].

Cysts in other organs. ADPKD is associated with cysts in other organs such as liver, spleen, pancreas, and seminal vesicles. However, extensive cystic disease in these organs is rare in childhood ADPKD, and routine screening is not recommended [56]. Extensive cystic involvement of the liver and/or pancreas should raise consideration for atypical or alternate disease including *GANAB* mutation in the case of liver cysts or *HNF1β* with significant pancreatic involvement. ADPKD-associated liver cystic disease is reported to be more severe in women who have had multiple pregnancies and in postmenopausal women receiving estrogen therapy [128]. The effect of hormonal contraception on liver cystic disease in young women with ADPKD is not known.

Intracranial aneurysms (ICA). ADPKD is associated with a fivefold higher prevalence of ICA than in the general population. This risk is particularly increased in patients with a family history of PKD-associated ICA or subarachnoid hemorrhage (up to 10% of the general population with ADPKD; 22% with positive family history versus 6% with negative family history) [110]. PKD-associated intracranial aneurysms are extremely unusual in childhood and routine screening is not recommended.

Prenatal, Very Early Onset (VEO), and Severe ADPKD

With advances in imaging resolution and increased application of imaging techniques from prenatal life onward, there has been an increase in the incidental diagnosis of ADPKD in childhood. By prenatal imaging, ADPKD may be evident in the fetus as enlarged kidneys, abnormal kidney echogenicity with increased corticomedullary differentiation (in contrast to the decreased corticomedullary differentiation more typical of ARPKD, and/or discrete cysts). The family history should be carefully reviewed, and in cases where a diagnosis is not evident, sonographic screening of parents may reveal previously undiagnosed ADPKD (or other relevant cystic kidney disease of ARPKD) and discrete cysts. The mere detection of such prenatal imaging abnormalities does not necessarily portend poor outcome, as serial monitoring in childhood is often reassuring, with enlarged kidneys improving to normal size over early childhood [9]. However, when accompanied by signs of fetal kidney dysfunction such as oligoanhydramnios, a high level of concern is warranted. Occasionally, significant unilateral kidney cystic involvement is observed in childhood and can be mistaken for other cystic kidney conditions before progression to involve both kidneys occurs [42]. Although all individuals with ADPKD are expected to develop kidney cysts, there can be significant variability even within the same family.

Importantly however, a subgroup of children has more severe ADPKD than the classical course and may suffer ESKD in early childhood [41, 97, 127]. In the most severe cases, oligoanhydramnios can lead to pulmonary hypoplasia and death in the neonatal period. For surviving children, hypertension can be severe and require multidrug therapy, and features of chronic kidney disease may be present from infancy. The term “very early onset” (VEO) ADPKD is used to distinguish this group of affected children who are diagnosed before 18 months of age due to enlarged kidneys accompanied by hypertension, persistent proteinuria, and/or reduced eGFR. A well-matched case control study found that VEO patients were much more likely to develop hypertension and to progress to eGFR ≤90 mL/min/1.73 m^2 during childhood as compared to their non-VEO counterparts [97]. All cases of early ESKD occurred in the VEO group. The frequency of glomerular hyperfiltration was similar between the groups but occurred earlier in the VEO group. The term “early onset” ADPKD has been suggested to identify children diagnosed with ADPKD between 18 months and 15 years of age with enlarged kidneys accompanied by hypertension, proteinuria, and/or reduced eGFR [33]. As noted previously, more recent studies have elucidated the role of biallelic and digenic variants as contributors to severity of disease in VEO and severe ADPKD.

Diagnostic Considerations

Diagnostic criteria for ADPKD in adults have been extensively defined [25] but are less well-established in children. Clinical features that raise suspicion of ADPKD in adults include: multiple bilateral kidney cysts in the absence of manifestations suggestive of an alternate cystic kidney disease; cysts in other organs such as liver, seminal vesicle, pancreas; kidney or liver enlargement; early onset hypertension before 35 years of age; intracranial aneurysm; family history of ADPKD. It is important to obtain a detailed family history of cystic kidney disease and associated complications. The diagnosis of ADPKD in adults is typically confirmed by imaging, with genetic testing reserved for atypical cases or to exclude ADPKD in a potential kidney donor. The diagnosis is established in a proband when age-specific ultrasound or MRI criteria are met in the setting of a family history of a first-degree relative with ADPKD or with identification of a heterozygous pathogenic variant in one of the genes known to cause ADPKD. Teenagers and young adults have been included in some of the studies which established the imaging criteria for ADPKD, but the majority of participants were older adults. Pei et al. [105] suggested that for individuals aged 15–39 years with an affected first-degree relative, the presence of three or more (unilateral or bilateral) kidney cysts establishes a diagnosis of ADPKD. The positive predictive value for this criterion is 100% for both *PKD1* and *PKD2*. However, the sensitivity is lower for *PKD2* (70% vs. 94% for PKD1). By MRI, in individuals aged 16–40 years with an affected first-degree relative, the finding of more than 10 cysts is sufficient for diagnosis of ADPKD [106]. While the identification of kidney cysts is obviously supportive of the diagnosis within these parameters, an absence of cysts is less informative. Because mild structural kidney disease can be seen with incompletely penetrant, non-truncating *PKD1* variants or pathogenic variants in other ADPKD-related genes, normal kidney imaging does not exclude the diagnosis of ADPKD in at-risk individuals under 40 years of age [105].

These guidelines therefore have important implications for elective screening of at-risk individuals, and the potential for a non-informative result should be reviewed with families before pursuing routine imaging screening in at-risk children.

The imaging criteria for diagnosis in earlier childhood are not as clearly established. Some have suggested that in the setting of an affected first-degree relative, a single kidney cyst is sufficient for diagnosis [56] while others have proposed that bilateral kidney cysts be required [115]. There are no established imaging-based criteria for diagnosis of ADPKD in children without a family history. In this setting, diagnosis is largely based on clinical phenotype with genetic screening available for confirmation. In the absence of known family history, it can be informative to obtain imaging on parents following the diagnosis in a child. De novo mutations can occur. As previously noted, genetic testing may also be particularly helpful in atypical presentations or in sporadic ADPKD without clear family history.

Regarding choice of imaging modality for diagnosis in childhood, TKV assessment appears to correlate well between MRI and ultrasound in childhood [16] but MRI is preferred when very accurate assessment of TKV is needed, as in the research setting. The correlation between standard ultrasound and MRI diminishes with more progressive structural disease due to both the user-dependent nature of sonographic kidney measurement and the invalid assumption of relatively normal kidney architecture using the ellipsoid formula to estimate sonographic TKV. However, the clinical utility of MRI for routine imaging may be limited in very young children due to cooperation and motion artifact. CT is also accurate for assessing structural kidney disease, but repeated CT is often avoided due to risks of radiation exposure. More recent studies suggest that 3D ultrasound with manual contouring may represent an alternative to MRI with improved accuracy versus 2D ultrasound in early ADPKD [11, 55].

Screening in asymptomatic at-risk minors. Before pursuing routine sonographic or genetic screening for asymptomatic minors at-risk for ADPKD due to known family history, a thoughtful discussion with parent(s) and patient (as developmentally appropriate) is warranted. As application of disease-specific therapies (e.g., pravastatin, tolvaptan; see "Treatment" section) evolves in pediatric ADPKD, more widespread routine screening is anticipated. However, families must understand the limitations, including the imperfect nature of radiographic screening to fully exclude ADPKD in childhood, and implications for psychological adjustment and insurability, and the minor should be involved in the decision to screen as developmentally able. In the absence of elective radiographic/genetic screening in asymptomatic minors, annual assessment for nephromegaly, hematuria/proteinuria, and hypertension is recommended [56]. In cases where screening is not pursued, parents should be counseled regarding their responsibility to inform their children of disease risk when they reach the age of majority.

Preimplantation genetic diagnosis (PGD). PGD for ADPKD is possible if a conclusive genetic mutation has been identified in the affected parent. In addition to the ethical implications, discussion of PGD should consider that although common, ADPKD in its typical form is not usually life-threatening or even life-limiting. However, specific genetic mutations (biallelic disease or truncating *PKD1* mutations) may have bearing in such discussions. One study found that while nearly 70% of affected CKD and ESKD participants felt that PGD should be offered to ADPKD patients, less than 20% of these same participants would consider termination of pregnancy for a diagnosis of ADPKD [131].

Differential diagnosis. The differential diagnosis of ADPKD includes numerous cystic kidney diseases. Historically ADPKD diagnosed in childhood was often mistaken for ARPKD due to the age at presentation; this was particularly common with VEO ADPKD. Prenatally, both conditions can be associated with enlarged echogenic kidneys and can be accompanied by oligoanhydramnios if significant kidney dysfunction is present. It has been suggested that increased corticomedullary differentiation (CMD) is more common in ADPKD while decreased CMD occurs more commonly with ARPKD. Macrocysts are more common in ADPKD but can occur with either condition; these often resolve early in life to a more typical microcystic appearance in ARPKD. Both conditions can occur in neonates or infants with severe hypertension, nephromegaly, and kidney failure. In the setting of an apparently negative family history, screening of parents for cystic kidney disease may be helpful. Sonographic imaging of the liver may also be helpful, as significant liver involvement is less likely to occur with a *PKD1* or −2 mutation. A genetic panel for cystic kidney diseases may be necessary in some cases to help differentiate these conditions (or combined mutations) and is of particular value for severe early presentation.

HNF1β-nephropathy is an autosomal dominant condition with widely varying phenotype which may include uni- or bilateral cysts, enlarged kidneys, hypodysplasia, or agenesis. Many patients are described with abnormal prenatal ultrasound with enlarged kidneys with increased echogenicity [34], similar to ADPKD or ARPKD, but there may be associated anomalies such as genital or pancreatic malformations. A family history of kidney or pancreatic disease, hyperuricemia, hypomagnesemia, or maturity onset diabetes of the young type 5 should raise particular concern for this diagnosis. Of note, however, 50% of cases carry de novo mutations with a negative family history. Genetic testing is needed to confirm diagnosis.

Simple kidney cysts are rarely seen in children but increase in frequency with age, with an incidence of less than 0.5% in children reported in 1991 [87] but more than 10% in adults aged 50 years and older [29]. With advanced imaging techniques and widespread application of various radiographic modalities in childhood, experience suggests that the incidence of simple cysts in childhood is higher than previously reported. By definition a simple cyst occurs in the kidney with otherwise normal parenchyma and a normal contralateral kidney. Simple cysts appear as round, thin-walled, anechoic, non-septated cysts, separate from the collecting system, with no Doppler blood flow [55]. Because single kidney cysts are rare in children and may be the first manifestation of an underlying pathologic cystic kidney disease, it is important to perform a detailed medical and family history and to pursue appropriate follow-up ultrasound examination to ensure no development of additional cysts or change in cyst size or imaging characteristics.

Tuberous sclerosis complex (TSC) is usually diagnosed based on clinical and other imaging features before significant kidney involvement is evident. Angiomyolipomata are the most common kidney manifestation of classic TSC and are also a major clinical diagnostic feature. Kidney cysts affect about 40% of children with classic TSC; the presence of multiple kidney cysts is considered a minor clinical diagnostic feature [72]. In classic TSC, kidney cysts are usually small and few in number. However, patients with the TSC2/PKD1 contiguous gene syndrome demonstrate a kidney phenotype associated with severe large bilateral cysts evident from early in life with associated severe kidney enlargement and high risk of progression to early ESRD [80].

Multicystic dysplastic kidney (MCDK) is characterized by replacement of the entire kidney with multiple disorganized cysts of varying size lacking any normal surrounding parenchyma. Such “kidneys” have no function. Thus, bilateral MCDK is associated with development of anhydramnios and anticipated perinatal death due to Potter sequence. In childhood, ADPKD does rarely present with asymmetric disease, but the severity of the unilateral MCDK’s architectural distortion and the lack of function by nuclear scan should help to differentiate these conditions. Renal cystic dysplasia is characterized by the finding of at least one cyst within an abnormal-appearing kidney, for example, hyperechogenicity, small kidney, loss of corticomedullary differentiation. The extent of structural kidney disease can be variable and can affect one or both kidneys. Such cystic dysplastic kidneys do not tend to be enlarged but rather normal to small in size and with more impressive parenchymal abnormality than typically observed between the cysts of ADPKD. Dilated calyces in patients with high-grade obstruction or urinoma can also be misdiagnosed as renal cysts. If concern exists, diuretic renal scan and urologic consultation are helpful [55].

Nephronophthisis is typically associated with normal-sized to small kidneys and bilateral increased echogenicity. Corticomedullary cysts can occur in nephronophthisis but in most cases are not evident until late in the course of the disease. The condition can manifest as isolated kidney disease or as part of a syndrome with associated manifestations in other organs. Extrarenal manifestations or deteriorating kidney function in the absence of extensive structural cystic kidney disease can help to differentiate nephronophthisis from ADPKD.

Cystic tumors are fortunately rare in childhood. Concerning signs of malignancy by ultrasound include a unilateral single cystic mass with a thickened wall, septations, and possibly calcifications, with enhanced perfusion on color Doppler imaging [55]. Numerous cystic lesions would be uncommon with kidney malignancy. More extensive imaging by CT or MRI to examine the lesion as well as assess abdominal lymph nodes and potential tumor infiltration into surrounding tissues or venous system is needed if malignancy is suspected.

Prognosis

Given the growing recognition that clinical manifestations and progressive structural kidney disease are occurring in children with ADPKD, it is critical to have a means to identify patients who are at the highest risk of disease progression in order to optimize individual treatment and to encourage appropriate participation in research trials. In this regard, studies have identified several factors which are associated with poor prognosis in adults with ADPKD. These include: onset of hypertension before 35 years of age; first episode of gross hematuria before 30 years of age [31]; hyperlipidemia and increased body mass index [99]; high urine sodium excretion [144]; decreased kidney blood flow [139]; large total kidney volume [24, 70]; and presence of a truncating PKD1 mutation [30, 66].

Such observations have led to the development and validation of prognostic tools to identify older adolescents and adult patients at highest risk for progression to ESKD. Irazabal et al. [70] formulated a risk prediction tool based on radiographic imaging from MRI/CT data from 590 ADPKD patents aged 15–80 years, with serum creatinine under 5 mg/dL within 6 months of imaging, and with at least two follow-up measurements of serum creatinine before reaching ESKD. The tool initially excludes atypical cases with marked asymmetry which are not suitable for the model and then stratifies the typical cases into five classes (A-E) based on growth rates (<1.5%, 1.5–3%, 3–4.5%, 4.5–6%, or >6% per year) estimated from patient age and theoretical starting htTKV of 150 mL/m. The model has been validated in other adult ADPKD cohorts and can be utilized to predict future GFR decline with reasonable accuracy. Patients in class 1C-1E are considered most likely to benefit from more aggressive or novel interventions. This imaging calculator can be used from the age of 15 years.

The Predicting Renal Outcome in Polycystic Kidney Disease (PROPKD) score was developed from 1341 ADPKD patients with a median age of 54.7 years and focuses on clinical and genetic parameters to stratify risk of kidney disease progression [31]. This score is typically applied from 35 years of age or older and assesses the presence of high-risk variables linked to age at ESKD onset including male gender [1 point], hypertension before 35 years of age [2 points], first urologic event before 35 years of age [gross hematuria, symptomatic cyst hemorrhage, cyst infections, flank pain due to cysts; 2 points], and genetic mutation (*PKD2* [0 points] versus non-truncating *PKD1* [2 points] versus truncating *PKD1* mutation [4 points]). With a scoring system from 0 to 9, the risk categories include low risk (0–3 points), intermediate risk (4–6 points), and high risk (7–9 points) of progression to ESKD, with corresponding observed median ages for ESKD onset of 70.6, 56.9, and 49 years, respectively. A score of 3 or below eliminated evolution to ESKD before 60 years of age with a negative predictive value of 81%, while a score greater than 6 predicted ESKD onset before 60 years of age with a positive predictive value of 90%. A modified version is available when genetic mutation is not known.

As yet no studies have delineated the factors occurring early in life which identify high-risk children with ADPKD, although research in this area is ongoing. Large registries may prove helpful in this regard. Recently, a new initiative has been launched to provide an observational evidence base for unified diagnostic, follow-up, and treatment approaches for ADPKD children. The ADPedKD registry is a World Wide Web-based database, including both retrospective and prospective longitudinal data from young ADPKD patients [35].

Treatment

Lifestyle measures. In recent years, much attention has focused on lifestyle measures which patients can implement to optimize health in

ADPKD. Given data suggesting a potential deleterious role of arginine vasopressin (AVP) in kidney cyst development, there has been interest in whether high water intake alone could suppress endogenous AVP concentration and thereby reduce kidney cyst growth [140]. The ease of access, minimal side effects, and minimal expense make this a highly desirable intervention if effective. Studies in adults have shown that water intake to target a goal urine osmolality below 280 mOsm/kg H_2O is attainable [148] and that the required daily water intake to achieve the urinary dilution goal can be reduced with a low-solute diet [2]. Longer randomized controlled trials to examine the impact of high water intake on TKV are in progress in ADPKD adults [153]. High fluid intake might also be generally beneficial to reduce the risk of nephrolithiasis and UTI in ADPKD. The CRISP study of polycystic kidney disease and a post-hoc analysis of the HALT-PKD clinical trials of renin-angiotensin blockade, both primarily consisting of adult ADPKD participants, suggested that higher dietary sodium intake was associated with faster growth of kidney cysts [141, 144]. Given this concern and the high risk of hypertension in pediatric ADPKD as compared to the general population, education for children and families on appropriate dietary sodium intake is worthwhile. With the rising prevalence of childhood obesity and its associated cardiovascular risk factors, targeting normal body mass index (BMI) is recommended for general health in children. Studies in adult ADPKD have also reported that BMI ≥ 30 kg/m^2 is associated with faster increase in TKV and decline in eGFR over time [99]. More recently interest has arisen in metabolic dysfunction underlying epithelial cell proliferation in ADPKD and whether dietary interventions such as daily caloric restriction, intermittent fasting, time-restricted feeding, or a ketogenic diet could be beneficial for metabolic reprogramming (reviewed in [101]). The relevance to and safety in affected children would require further study. As cardiovascular disease is the most frequent cause of mortality in ADPKD, routine monitoring and appropriate intervention for hyperlipidemia and avoidance of smoking/vaping are reasonable recommendations. Avoidance of potential nephrotoxins whenever possible including nonsteroidal anti-inflammatory drugs is also appropriate.

Blood pressure control. Controlling hypertension is a critical aspect of chronic kidney disease management in both children and adults. Lifestyle counseling with respect to appropriate dietary sodium restriction, routine activity, and targeting normal body mass index is indicated as the initial measure and is an important component of anticipatory guidance for at-risk individuals and those diagnosed with ADPKD, both with and without hypertension. When pharmacologic treatment is necessary, blockade of RAAS is usually the preferred mechanism due to concern for RAAS activation as a major contributor to ADPKD-related hypertension and effect to decrease chronic proteinuria and glomerular hyperfiltration.

Although multiple studies have suggested a role for aggressive control of hypertension to delay deterioration in kidney function in adults and children with chronic kidney disease [40, 74], the optimal level of blood pressure control in pediatric ADPKD is not well established. A five-year interventional trial in ADPKD children and young adults ages 4–21 years was conducted to examine the effect of aggressive blood pressure control with ACEI in participants with hypertension (blood pressure above the 95th percentile for height, sex, and age; randomized to ACEI treatment with enalapril with goal blood pressure at the 90th versus 50th percentile) and participants with high normal blood pressure (75th to 95th percentile for height, sex, and age; randomized to either enalapril with goal blood pressure at the 50th percentile or to observation without treatment unless hypertension developed) [15]. Regardless of the degree of hypertension control, participants in both hypertensive groups had a significant increase in TKV and decrease in eGFR over the study period. No significant differences were observed in LVMI or urine albumin excretion between study groups or over time. These results emphasized the high risk for structural and functional kidney disease progression associated with established hypertension, even in ADPKD children. With high normal blood pressure, both study groups also demonstrated significant increase in TKV over time. However, participants randomized to observation showed a decline in eGFR and increase in LVMI over time while these parameters were stable in the ACEI-treated group. These findings suggested that children and young adults with ADPKD and high normal blood pressure may represent a subgroup for whom rigorous control of blood pressure with ACEI could provide kidney functional and cardiovascular benefit. Important limitations of the clinical trial included a relatively small number of participants as compared to adult trials despite recruitment across the United States, suboptimal achievement of blood pressure goals in the aggressively treated hypertensive group, and a generous dropout rate (27% overall).

Subsequently, the multicenter HALT-PKD trial was conducted [124]. This double-blind, placebo-controlled trial included 558 hypertensive participants 15–49 years of age, with early stage ADPKD (eGFR >60 mL/min/1.73 m^2) who were randomized to either a standard blood pressure target (120/70–130/80 mmHg) or to a low blood pressure target (95/60–110/75 mmHg) and to either the ACEI lisinopril plus the angiotensin receptor blocker telmisartan or to lisinopril plus placebo. Serial follow-up was performed for 5–8 years with a primary outcome of annual percentage change in TKV by MRI. The trial included a very limited number of pediatric participants, with mean age of study participants overall of 36 ± 8 years. Combined lisinopril/telmisartan did not significantly impact the rate of increase in TKV as compared to lisinopril/placebo. However, as compared to standard blood pressure control, the rigorous blood pressure control group demonstrated a small (1.1% per year) but statistically significant reduction in TKV and no change in eGFR. Of note, there was no control arm without RAAS, limiting the ability of this trial to assess the specific effect of RAAS blockade on TKV. Limitations of the study included a higher frequency of PKD2 mutations in the low BP group and a limited number of participants reaching blood pressure goals on a consistent basis. Based on the trial, aggressive control of blood pressure was recommended for this group of patients.

The KDIGO Controversy Conference on ADPKD [25] advised a target blood pressure of less than 140/90 in adults with ADPKD with a stricter goal below 130/80 if macroalbuminuria was present. They further recommended management of hypertension in ADPKD children per routine guidelines for the general pediatric population (goal below the 90th percentile for age, sex and height) and supported RAAS blockade as the preferred first-line treatment for hypertension in ADPKD. More recently, international consensus guidelines specific to pediatric ADPKD [56] recommended at least annual blood pressure measurement for children at-risk of or diagnosed with ADPKD with a preference for periodic 24-hour ambulatory blood pressure monitoring due to the increased risk of masked hypertension and advised blood pressure goal below the 75th percentile or $< 125/72$ mmHg for age greater than 16 years. Further, they noted that lowering blood pressure below the 50th percentile or $< 120/70$ mmHg if over 16 years of age might provide additional long-term benefit for hypertensive children with ADPKD, although with limited firm evidence to support this conclusion. Thus it seems reasonable to target 50th–75th percentile blood pressure goals for hypertensive children with ADPKD.

Pharmacologic therapy for ADPKD. With advances in the understanding of the mechanisms which contribute to kidney cyst development, the last two decades have seen a major increase in interventional clinical trials, particularly in early stage ADPKD, designed to mitigate progressive development of kidney cysts and thereby slow progression to ESKD (Fig. 1).

mTOR inhibitors. Inhibitors of the mechanistic target of rapamycin (mTOR) have been utilized in clinical trials of adult ADPKD with disappointing results. The mTOR pathway has an important role in cell growth and proliferation, including interactions of the cytoplasmic tail of polycystin-1 with tuberin. Animal studies suggested that rapamycin, an inhibitor of mTOR, reduced cystogenesis in a dose-dependent fashion [157]. Unfortunately, however, sirolimus treatment had no impact on TKV or kidney function in an 18-month clinical trial in adults with ADPKD with normal kidney function [126]. Gastrointestinal side effects were common, with 82% of treated participants complaining of mucositis. Similarly, everolimus treatment for

2 years in adults with ADPKD resulted in a nonsignificant slower rate of increase in TKV and faster decline in eGFR [147]. Mucositis and cytopenias were common in the treatment group. For both studies, concern was raised that appropriate local (i.e., kidney cyst) concentrations of drug were not achieved before systemic side effects prevailed. These medications have not been studied in pediatric ADPKD but other than TSC2/PKD1 contiguous gene syndrome, use is likely to be limited by the high rate of side effects and concern for potential adverse effects of long-term immunosuppression.

Somatostatin analogs. Somatostatin analogs including octreotide and lanreotide have been attempted in adults with ADPKD to manage both polycystic kidney and polycystic liver disease. Such medications inhibit cAMP production via stimulation of Gαi. The ALADIN trial reported decreased rate of growth in TKV after 1 year of treatment with octreotide, but the effect was not sustained at 3 years of study [18, 111]. An observational study of 6 months' treatment with lanreotide in adults with polycystic liver and kidney disease demonstrated a statistically significant but clinically minimal decrease in median TKV (1023 to 1012 mL; $p = 0.0006$) [54]. These medications may have a more valuable role in management of PKD-associated polycystic liver disease, which is not a feature of childhood ADPKD.

Statin therapy. HMG–CoA reductase inhibitors (statins) improve kidney blood flow and eGFR and attenuate inflammation through vascular and glomerular nitric oxide production [86]. Lovastatin treatment reduced the severity of functional and structural kidney disease in an animal model of ADPKD [158] with subsequent studies attributing these effects to statin-mediated reduction in arachidonic acid-mediated inflammatory markers, normalization of metabolism of nitric oxide precursors, and improvement in kidney energy cell metabolism [76]. In a prospective, double-blind, randomized controlled clinical trial of 110 children and young adults aged 8–22 years with ADPKD and normal kidney function, treatment with pravastatin resulted in a significantly slower increase in htTKV over time as compared to placebo (23 ± 3 vs. 31 ± 3% per 3 years; $P = 0.02$) [17]. All participants were treated with lisinopril with target blood pressure at the 50th to 75th percentile. As anticipated, eGFR did not differ between the groups. LDL and total cholesterol levels were not directly correlated with clinical outcome variables including htTKV, albuminuria, or LVMI. Pravastatin therapy was associated with reduction in plasma concentrations of cyclooxygenase- and lipoxygenase-derived plasma lipid mediators known to enhance the pro-fibrotic effects of angiotensin II and to activate the peroxisome proliferator–activated receptor ɣ (PPARɣ) which has been implicated in cyst growth [77]. The medication was well-tolerated in the cohort. Subsequently, a secondary analysis of the HALT-PKD clinical trial described no effect of self-reported statin use versus no statin use on TKV in adults with ADPKD [12]. It is important to note that statin use was not randomized or controlled in the HALT-PKD trial and that the study participants also had significantly more advanced disease than the pediatric population described above. Whether an antiproliferative effect of statins is more impactful early in the course of ADPKD, as has been observed with statins and renal hemodynamics [159], remains to be seen. In this regard, a controlled clinical trial in adults with early-stage ADPKD is ongoing (NCT03273413).

Vasopressin V_2 receptor blockade. In 1989, Grantham et al. reported that in contrast to normal human kidney cells, ADPKD cells demonstrated vasopressin-induced cAMP-stimulated chloride-driven fluid secretion, bringing focus to cAMP as a critical regulator of kidney cyst growth in ADPKD and potential therapeutic target [59, 89]. Several years later, pharmacologic vasopressin V_2 receptor antagonists became available. Treatment with such agents was associated with a marked decrease in cyst fluid accumulation in several rat and mouse models of polycystic kidney disease [52, 138, 150]. These studies formed the basis of the multicenter international TEMPO 3:4 clinical trial which included nearly 1500 adults aged 18–50 years with ADPKD, eGFR >60 mL/min per 1.73 m^2, and large kidneys (TKV ≥750 mL) who were randomized to vasopressin V_2 receptor blockade with tolvaptan or to placebo for 3 years

[142]. Participants receiving tolvaptan demonstrated slower annual increase in TKV (2.8 vs. 5.5%; $P < 0.001$) and slower annual decline in kidney function (-2.6 vs. -3.8 mg/mL; $P < 0.001$) than controls. Suppression of plasma AVP was observed in both groups and was attributed to voluntary water intake by placebo participants. It is important to note that these trial participants had relatively advanced structural kidney disease and thus the magnitude of benefit to the general and more specifically to the pediatric ADPKD population requires further study. Subsequently, tolvaptan was approved for rapidly progressive ADPKD in adults in several countries around the world. There is some variability in the definition of "rapidly progressive," and several panels have published statements in this regard [27, 50, 67]. Of note, there is concern for potential hepatotoxicity with tolvaptan which can be severe and irreversible in some cases.

The therapeutic potential of tolvaptan in patients at risk of early disease progression might be even greater at pediatric age, when the kidney has not yet been irreversibly damaged by cyst growth [122]. A phase 3 study evaluating tolvaptan over 3 years in children and adolescents with ADPKD is ongoing. This trial aims to evaluate the pharmacodynamic properties, safety, and efficacy of tolvaptan in this young population. In addition, lixivaptan, a nonpeptide V_2R antagonist with potentially less hepatotoxicity [155], will be evaluated in a phase 3 adult trial that will start recruiting patients in 2021.

Curcumin. Curcumin is found in the spice turmeric and is a naturally occurring polyphenol which has the ability to activate transcription of antioxidants, suppress inflammation, and reduce cell proliferation, leading to its application in several conditions of abnormal cell growth. It has been shown to mitigate acute and chronic kidney injury, possibly by diminishing activation of the pro-inflammatory transcription factor NFkB and its downstream target tumor necrosis factor-α (TNF-α) as well as enhancing the anti-inflammatory transcription factor PPARɣ [84]. Through effects on intracellular signaling proteins, curcumin has been shown to reduce kidney cyst growth in vitro in a dose-response manner [51]. In the Pkd–1 deletion mouse model, curcumin treatment improved kidney histology and cystic proliferation [82]. Curcumin also has effects on vascular function which have been attributed to increased vascular nitric oxide bioavailability and arterial eNOS expression and reduced vascular oxidative stress [44, 95]. As noted previously, vascular dysfunction is a common derangement associated with ADPKD in both children and adults and is thought to increase long-term risk of cardiovascular disease. Upon this background, a randomized placebo-controlled interventional trial of curcumin therapy was conducted in children and young adults with ADPKD [100]. The study assessed brachial artery flow-mediated dilation and aortic pulse wave velocity at baseline and following 12 months of curcumin treatment versus placebo. Secondary outcome measures included htTKV as well as urine and plasma markers of inflammation and oxidative stress. Results are anticipated in late 2021.

AMPK activation. An alternate approach to suppress mTOR activity is via stimulation of AMP-activated kinase (AMPK) [92]. In this regard, metformin, which is a pharmacological activator of AMPK, has been demonstrated to arrest cyst growth in mouse models of PKD [132]. The medication is relatively inexpensive, has widespread use in diabetes mellitus and polycystic ovary syndrome in both adults and children, and may have some anti-inflammatory and anti-fibrotic effects which could be of potential benefit for vascular dysfunction in ADPKD. Clinical trials are ongoing in adult ADPKD.

Tyrosine kinase inhibitors. Epidermal growth factor (EGF) receptor is known to be overexpressed and mislocalized to the apical membrane in both ADPKD and ARPKD [38, 116]. Tyrosine kinase inhibitors that block EGF receptor activity and its downstream signaling have been shown to slow disease progression in rodent models of PKD [130]. A phase 2, randomized placebo-controlled multicenter study of the oral dual Src/Bcr-Abl tyrosine kinase inhibitor bosutinib was undertaken in adults with ADPKD with relatively preserved eGFR and large kidneys (TKV $\geq$ 750 ml) [135]. Bosutinib was associated with a reduction in annual rate of kidney enlargement (0.84% vs. 4.74%) as compared with controls, with similar annualized eGFR decline. However, there was a high rate of bosutinib-related gastrointestinal and liver adverse events including the need for permanent discontinuation of bosutinib in several participants. Such findings raise concern for the generalized effect of tyrosine kinase inhibitors on proliferating cells when more targeted impact on kidney cyst epithelium is desired. Safety and tolerability may thus limit the application of these medications in the near future to pediatric ADPKD.

Others. Venglustat is an inhibitor of the enzyme glycosylceramide synthase (GCS) which catalyzes an early step in the synthesis of many glycolipids. GCS inhibition has been shown to reduce cystogenesis, improve urinary concentration, and reduce the rate of renal functional decline in three animal models of polycystic kidney disease including the orthologous ADPKD mouse [96], suggesting a potential role for compounds like venglustat in patients with ADPKD. A phase 1 trial in healthy volunteers suggested a favorable safety and tolerability profile for venglustat [108], and a multicenter, randomized, double-blind, placebo-controlled study to examine the efficacy and safety of venglustat on TKV growth and eGFR decline in adult patients at risk for rapidly progressive ADPKD is now in progress (STAGED-PKD; NCT03523728). Bardoxolone exerts antioxidant activity by promoting the activation of nuclear factor erythroid2 derivative (Nrf2) and downregulation of the proinflammatory NF-kB. In renal cortical collecting duct cells, tolvaptan has been shown to activate the Nrf2 antioxidant pathway through a different mechanism than bardoxolone, suggesting potential for synergistic effects [45]. A multicenter, randomized, double-blind, placebo-controlled phase 3 clinical trial evaluating the safety, tolerability, and effect of bardoxolone on eGFR decline in adults with ADPKD is underway (FALCON; NCT03918447).

Conclusion

The recent progress in understanding the pathophysiology and disease progression in ADPKD led to the approval of tolvaptan as the only disease-specific medication for ADPKD. However, with its limited therapeutic achievements and severe side effects, there is still an unmet need to identify efficacious drugs. Because deterioration of renal function is not observed until the destruction of at least 50% of the parenchyma, therapies implemented at later stages are less likely to be successful since the patient may already have reached a point of no return. Consequently, research to identify new biological targets and surrogates for disease progression are strongly required as children and young adults with ADPKD may represent the critical therapeutic window to reduce future renal and cardiovascular risk. Walking in this road of progress, the ultimate goal of the pediatric community should be to empower patients and families to make their own informed decisions, to advise follow-up and treatment of ADPKD children with early manifestations, and to monitor at-risk children for disease manifestations such as hypertension and proteinuria. Treatment of these disease-modifying factors and improving the lifestyle early in ADPKD might impact the long-term outcome. The World Wide Web-based ADPedKD registry initiative demonstrated the strong collaborative commitment of the pediatric community to improve this gap by raising awareness of this disease and will help to generate further observational evidence for clinical and therapeutic recommendations.

Cross-References

- ▶ Autosomal Dominant Tubulointerstitial Kidney Disease
- ▶ Autosomal Recessive Polycystic Kidney Disease
- ▶ Evaluation and Management of Hypertension in Children
- ▶ Nephronophthisis and Related Ciliopathies
- ▶ Pathophysiology and Epidemiology of Hypertension in Children
- ▶ Renal Involvement in Tuberous Sclerosis Complex

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Autosomal Recessive Polycystic Kidney Disease

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Max C. Liebau and Lisa M. Guay-Woodford

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Abstract

Autosomal recessive polycystic kidney disease (ARPKD) is a monogenic disorder that primarily involves mutations in the *PKHD1* gene, although rare, atypical forms of ARPKD due to mutations in other genes have recently been described. For years, pediatric nephrologists have directed the clinical management of these patients. However, there is increasing recognition that ARPKD has multisystem effects and a comprehensive care strategy requires a multidisciplinary team. In severely

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affected infants, the diagnosis is often first suspected by obstetricians based on detection of enlarged, echogenic kidneys and oligohydramnios on prenatal ultrasounds. Neonatologists are central to the care of these infants, who may have respiratory compromise due to pulmonary hypoplasia and massively enlarged kidneys. Variability in the clinical expression of ARPKD liver disease requires pediatric hepatology evaluation and management. Surgical considerations range from the advisability of nephrectomy to relieve mass effects, placement of dialysis access, and kidney and/or liver transplantation. Families of patients with ARPKD also face decisions regarding genetic testing of affected children, testing of asymptomatic siblings, or consideration of preimplantation genetic diagnosis for future pregnancies. These considerations require the expertise of genetic counselors, geneticists, and reproductive endocrinologists. This chapter will discuss the clinical and genetic diagnosis ARPKD, current insights into the genetics and pathobiology of this disorder, management of organ-specific complications, and close with considering future directions for prognostic prediction of disease outcomes and the development of targeted therapies.

Keywords

ARPKD · ADPKD · *PKHD1* · FPC · Hepatorenal fibrocystic diseases

Introduction

Polycystic kidney diseases (PKD) are among the most frequent genetic causes of chronic kidney failure in children and adults. Autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) are cilia-related disorders characterized by fibrocystic hepatorenal changes and variable systemic manifestations [1]. Emerging evidence suggests while these disorders are phenotypically distinct, there is mechanistic overlap in their pathobiology. This chapter focuses on ARPKD, the rarer and often more severe form of PKD that typically presents antenatally or in early childhood [2, 3]. With its attendant significant morbidity and mortality, ARPKD is widely considered to be one of the most challenging disorders to manage, even in in the most advanced pediatric centers [4, 5].

Epidemiology, Diagnosis, and Pathology

ARPKD is a rare disease with an estimated incidence of 1 in 20,000 live births and carrier frequency of 1:70 [1, 4, 6]. Variability in the reported incidence is likely a consequence of multiple factors including higher disease frequency in isolated populations [7, 8], variation in inclusion criteria, and the confounding impact of genetically distinct diseases that can phenocopy the clinical presentation of ARPKD [9]. A recent study in the USA based on electronic health record (EHR) data calculated an annualized incidence of 1:26,485 live births [10].

ARPKD is mainly caused by variants in the gene *polycystic kidney and hepatic disease 1* (*PKHD1*) on chromosome 6 [11, 12]. In addition, variants in the gene *DAZ interacting zinc finger protein 1 like* (*DZIP1L*) on chromosome 3 have been identified in a few patients with an ARPKD phenotype [13]. Details on the genetics are discussed later in this chapter.

The clinical diagnosis of ARPKD is suspected according to the modified Zerres criteria: typical kidney imaging findings in addition to one or more of the following: (1) typical liver imaging, clinical or laboratory findings consistent with congenital hepatic fibrosis, (2) liver pathology showing biliary ductal plate malformation, (3) negative standard ultrasound findings in both parents or characteristic findings on high-resolution ultrasonography, and (4) diagnosis of ARPKD in an affected sibling by either pathology or genetics [14, 15]. In highly resourced countries, antenatal suspicion of ARPKD is raised by detection of enlarged, diffusely echogenic kidneys on routine antenatal screening [5, 16].

After birth, sonography typically reveals bilaterally enlarged, reniform kidneys with poor corticomedullary differentiation due to the ubiquitous microcysts that typically start in the medulla and radiate into the cortex [5, 17–19]. Typical images of kidney and liver disease are presented in Fig. 1. ARPKD has long been described as having a “salt and pepper” pattern on renal sonography. However, this pattern is now commonly observed among a range of ciliopathy-related renal cystic diseases [20]. On high-resolution ultrasound, diffuse corticomedullary involvement was associated with poorer renal outcome as compared to predominant medullary involvement [19]. Bilateral pelvicaliectasis and calcifications in the kidney have also been reported in ARPKD patients [6, 21].

Macrocysts may develop during the course of the childhood and thus, distinguishing ARPKD and other cystic kidney disorders such as HNF1B-nephropathy or early onset ADPKD may be difficult [17, 19]. Unlike in ADPKD, kidney length in ARPKD does not inexorably increase and renal growth may slow or kidney size contract as children age [22]. While total kidney volume (TKV) is a well-established prognostic marker in ADPKD [23], its prognostic value in ARPKD is less clear. There is no clear correlation between kidney length and renal function or length of the disease course [18]. However, one study reported a limited inverse correlation between body-surface-adjusted total kidney volume and renal function in an ARPKD cohort [24].

Different histopathological patterns have been described that may depend on the patient age and the extent of renal cystic disease. Typically, fusiform dilatations of the tubules and collecting ducts show radial expansion from the medulla to the cortex [25] (Fig. 2a–c). These dilatations are lined with columnar or cuboidal epithelium and remain in continuity with the full nephron [1]. A transient phase of proximal tubular cyst formation has been described in the fetal kidney as well as various mouse PKD models, but proximal tubular cysts are absent at birth in human ARPKD [25–27]. Dysplastic elements are not observed in ARPKD kidneys.

In addition to the cystic transformation of the epithelia-lined tubules, there is progressive interstitial fibrosis in ARPKD kidneys. A study of 54 fetuses following medical termination of pregnancy and 20 neonates who died perinatally found that the severity of cortical collecting duct dilatations, cortical tubular and glomerular lesions, and renal cortical and hepatic portal fibrosis increased with gestational age [25]. Moreover, the severity of cortical tubular lesions, glomerular lesions, and cortical fibrosis significantly correlated with the extent of collecting duct dilatation. Dilatations of the proximal tubule were observed almost exclusively prior to 30 weeks gestational age.

The ARPKD liver lesion is characterized by hepatobiliary changes that arise from the “ductal plate malformation,” in which defective remodeling of the developing biliary tree and the associated portal vein ramifications results in congenital hepatic fibrosis (CHF) [28, 29]. Biliary ectasia and dilatations can affect both intra- and

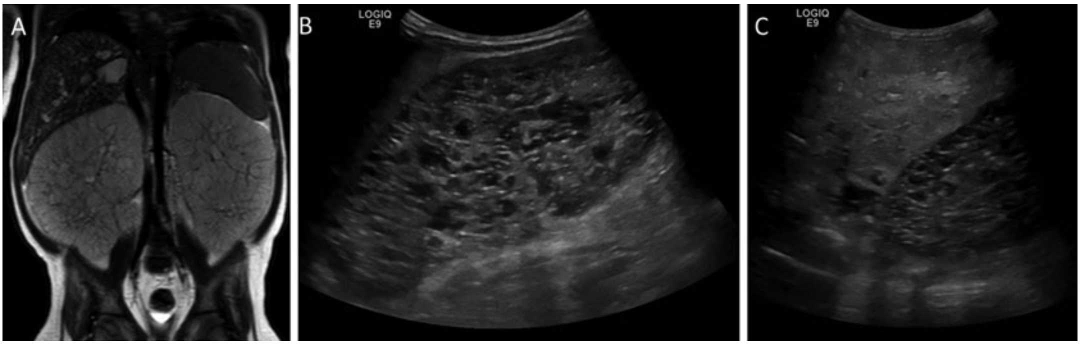

Fig. 1 Radiological features of ARPKD. Typical radiological features of ARPKD kidney and liver disease with massively enlarged and cystic kidneys as detected by coronal MRI (**a**) or ultrasound (**b**). Also note the dilated bile ducts in the MRI. Ultrasound shows inhomogeneous liver parenchyma during the course of the disease (**c**)

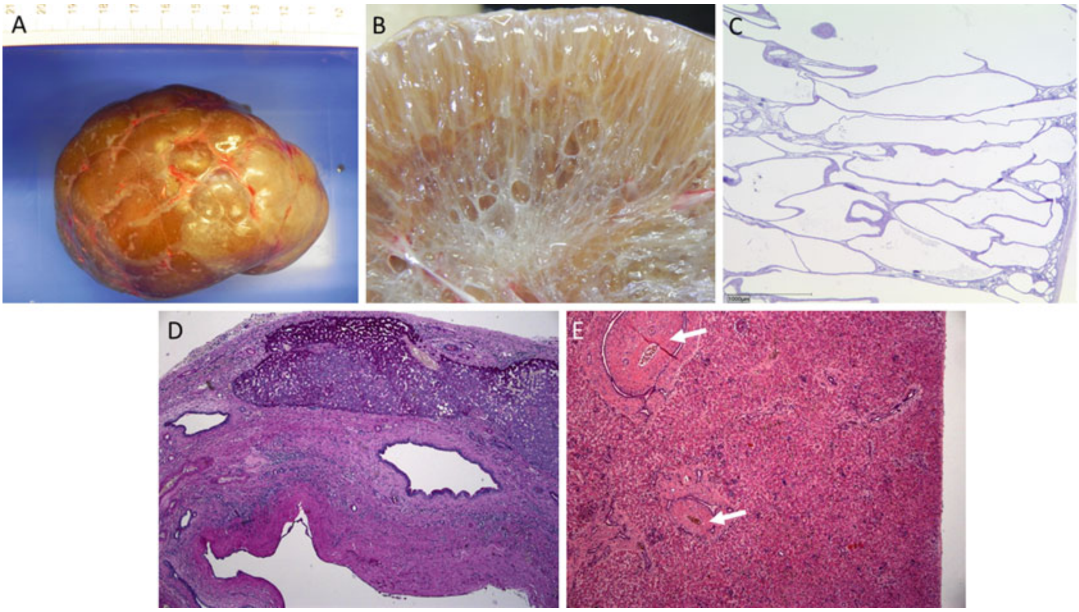

Fig. 2 Histopathological features of ARPKD. Macroscopic appearance of enlarged reniform kidneys (**a**) with ubiquitous tubular microcysts (**b**, **c**). Liver histology shows dilated bile ducts in an example of Caroli's syndrome (**d**) and the typical appearance of ductal plate malformation (**e**)

extrahepatic bile ducts. The most severe expression, involving both dilated bile ducts and congenital hepatic fibrosis, is termed Caroli's syndrome (Fig. 2d–e). ARPKD liver disease is characterized by progressive portal fibrosis, often with the development of fibrous septae that isolate normal liver parenchyma with functioning hepatocytes. Clinically, these histopathological features can result in portal hypertension and increased risk for cholangitis, while hepatocellular function (and thus serum transaminase levels) remains within the normal range [29–32].

Genetics

Molecular Basis of Disease

All typical forms of ARPKD are caused by mutations in *PKHD1*, which is among the largest human disease genes extending over 470 kb in the genomic sequence. Of the predicted 86 exons in *PKHD1*, 67 comprise the longest open reading frame which encodes fibrocystin/polyductin (FPC), a 4074 amino acid protein [12]. The gene appears to be transcriptionally complex, perhaps more so in the mouse than in humans [12, 33]. More than 700 pathogenic *PKHD1* mutations have been catalogued in the ARPKD mutation database (http://www.humgen.rwth-aachen.de) [34]. These variants have been identified along the entire length of the gene and approximately half are missense changes. While there is no apparent mutational hot spot, a missense mutation in exon 3, c.107C>T (p.Thr36Met), has been observed in a number of unrelated patients and accounts for approximately 15–20% of all mutant alleles [35].

Genotype-Phenotype Correlations

Multiple studies have examined ARPKD cohorts for potential genotype-phenotype correlations. Most patients are compound heterozygotes for *PKHD1* mutations and the functional effect of any particular mutant allele can be difficult to discern. Nevertheless, some broad themes are emerging. Notably, patients with two truncating mutations typically have a severe phenotype leading to perinatal or neonatal mortality, whereas missense mutations are typically associated with neonatal survival [9, 16, 35, 36]. No substantial clinical differences have been reported between patients with two missense mutations and patients with missense and truncating mutations *in trans*, suggesting that the missense mutation defines the disease severity (reviewed in [9]). In a recent study, the European ARegPKD Consortium found that the position of *PKHD1* variants is important in determining the phenotype. Patients with two missense variants affecting FPC amino acids 709–1837 or a missense variant in this region combined with a null variant less frequently develop end-stage kidney disease (ESKD). Patients with missense variants affecting FPC amino acids 1838–2624 have less severe liver disease, whereas variants affecting amino acids 2625–4074 are associated with poorer hepatic outcome [37].

However, there are notable exceptions. For example, children homozygous for *PKHD1* deletions or truncating mutations have survived well past the neonatal period [38, 39]. Moreover, not all missense mutations lead to a relatively less severe outcome, suggesting that missense variants have diverse impact on protein function [35]. Genetic and perhaps environmental modifiers likely also play a significant role in disease expression, as illustrated by significant phenotypic variability in subsets of families [40]. Taken together, these genetic features pose particular challenges for genetic counseling of ARPKD families.

Atypical ARPKD and Phenocopy Disorders

ARPKD is generally considered a genetically homogeneous disease. However, a recent report described seven children from four unrelated, consanguineous families with an atypical form of ARPKD that results from mutations in a second gene, *DZIP1L* [13]. The children presented with hypertension and large, cystic kidneys; some progressed to ESKD. A distinguishing feature of this ARPKD phenotype is the more moderate renal disease and the absence of clinically apparent liver disease.

The *DZIP1L* gene spans about 53 kb and encodes a 767 amino acid ciliary transition zone protein, DAZ interacting zinc finger protein 1 like (DZIP1L). Among the four reported families, two families had different homozygous missense changes predicted to be pathogenic, while the other two had distinct, homozygous truncating mutations. While the function of DZIP1L is largely unknown, it is implicated in hedgehog signaling and ciliogenesis.

The *cpk* mouse carrying a spontaneous truncating mutation in *Cys1* has been the most widely studied mouse model of ARPKD [41]. However, the relevance of this mouse model for human disease has been unclear. A recent report describes the first case of human ARPKD due to homozygosity for a *CYS1* variant, c.818+5G>A, which is predicted to disrupt splicing [42]. While it is surprising that *CYS1* deficiency causing ARPKD has been observed in only one family, this small gene has an extensive GC-rich region in its first exon. Such GC-rich regions can be difficult to amplify and sequence using Sanger sequencing and even next-generation sequencing [43].

ARPKD is the prototype of the hepatorenal fibrocystic diseases (HRFD; Table 1), a subset of recessively transmitted cilia-related disorders (ciliopathies) that are characterized by renal cystic disease and can be associated with congenital hepatic fibrosis and/or Caroli syndrome, as well as extrarenal manifestations [44]. These rarer disorders in which the kidney disease can phenocopy ARPKD include: nephronophthisis (MIM 256100), Joubert syndrome (MIM 213300), Bardet-Biedl syndrome (MIM 209900), Meckel-Gruber syndrome (MIM 249000), and oro-facial-digital syndrome I (MIM 311200). As with FPC and DZIP1L, the proteins disrupted in these disorders are critical in the structure/function of the primary apical cilium [41].

Finally, a form of PKD that can mimic either ARPKD or ADPKD and is associated with hyperinsulinemic hypoglycemia (HIPKD) has been described in 11 pedigrees, who had biallelic mutations in *PMM2,* which encodes phosphomannomutase 2 [45]. PMM2 is a key enzyme in N-linked glycosylation, and recessive mutations in *PMM2* are usually associated with congenital

Table 1 ARPKD and other hepatorenal fibrocystic diseases

Disease	Gene(s)	Renal disease	Hepatic disease	Associated features
ARPKD	*PKHD1; DZIP1L: CYS1*	Collecting duct dilatation	CHF[a]; Caroli syndrome	Growth retardation
ADPKD	*PKD1; PKD2; GANAB*	Cysts along entire nephron	Biliary cysts; CHF	Minimal in children
Nephronophthisis (NPHP)	*NPHP1-NPHP20*	Cysts at the corticomedullary junction	CHF	Tapetoretinal degeneration; situs inversus
Joubert syndrome (JBTS)	*JBTS1-JBTS37*	Cystic dysplasia; NPHP	CHF; Caroli syndrome	Cerebellar vermis hypo/aplasia with episodic hyperpnea; abnormal eye movements; intellectual disability
Bardet-Biedl syndrome (BBS)	*BBS1-BBS21*	Cystic dysplasia; NPHP	CHF	Retinal degeneration; obesity; post-axial polydactyly; hypogonadism in males; intellectual disability
Meckel syndrome (MKS)	*MKS1-MKS13*	Cystic dysplasia	CHF	Occipital encephalocele; polydactyly
Oral-facial-digital syndrome, type I (OFD1)	*CXORF5*	Glomerular cysts	CHF (rare)	Malformations of the face, oral cavity, and digits
Glomerulocystic disease	*PKD1; TCF2; HNF1B*	Enlarged; normal or hypoplastic kidneys	CHF (with *PKD1* mutations)	Diabetes; hyperuricemia
Jeune syndrome (short-rib thoracic dysplasia [SRTD])	*SRTD1-SRTD20*	Cystic dysplasia	CHF; Caroli syndrome	Short stature; skeletal dysplasia; small thorax; short limbs; polydactyly; hypoplastic pelvis
Renal-hepatic-pancreatic dysplasia (RHPD)	*NPHP3; NEK8*	Cystic dysplasia	Intrahepatic biliary dysgenesis	Pancreatic cysts, dysplasia, and/or fibrosis; splenic abnormalities; situs inversus
Zellweger syndrome	*PEX1–3;5;6;10;12–14;16;19;26*	Renal cortical microcysts	Intrahepatic biliary dysgenesis	Hypotonia; seizures; agenesis/hypoplasia of corpus callosum; characteristic facies; skeletal abnormalities; neonatal death

Adapted from O'Connor et al. [41]

[a] CHF – congenital hepatic fibrosis

disorder of glycosylation type 1a (CDG1A), which has a severe, pleiotropic phenotype.

Genetic Testing

Molecular diagnostic analysis is rapidly becoming the gold standard for diagnosing ARPKD and distinguishing this disorder from the large number of phenocopy disorders or syndromes [9]. Available data indicates that compound heterozygous or homozygous *PKHD1* mutations have been identified in approximately 80% of patients suspected of having ARPKD, with phenotypes ranging from perinatal demise to disease presentation in young adults. At least one *PKHD1* mutation has been found in more than 95% of families screened [9]. However, in patients with only one *PKHD1* variant or without any detectable *PKHD1* mutations, phenocopy disorders, e.g., due to mutations in *PKD1* or *HNF1B*, should be considered [41].

In practice, screening a panel of genes rather than analysis of *PKHD1* alone has become the preferred diagnostic approach [9]. Advances in next-generation sequencing (NGS) now enable simultaneous analysis of a large number of genes in a single test at relatively low cost. While whole exome or whole genome-based screening may become the standard for genetic testing in the near future, at present, targeted NGS panel testing is the most efficient diagnostic approach. Whichever methodology is used, the testing strategy should be designed to detect copy number variations (CNVs) such as heterozygous deletions (for example, in *HNF1B*) and to cover complex genomic regions, such as *PKD1*. Clinical genetic testing laboratories are summarized at the NIH-sponsored Genetic Testing Registry, www.ncbi.nlm.nih.gov/gtr.

Preimplantation Genetic Diagnosis (PGD)

In families who have had a previous child with severe ARPKD, the high recurrence risk (25%) often prompts interest in either prenatal diagnosis or PGD for future pregnancies. PGD requires prospective identification of the *PKHD1* mutations transmitted from each parent. The couple then must undergo in vitro fertilization. The resulting embryo is biopsied, with removal of 1–2 embryonic cells for genetic testing. PGD has successfully guided the birth of unaffected infants in at risk families [46].

Protein Function and Pathophysiology

FPC, the product of the *PKHD1* gene, is a 4074 amino acid, single-membrane spanning protein with a long extracellular N-terminus and short cytoplasmic C-terminus, that appears to have multiple isoforms [33]. During fetal development, *PKHD1* is broadly expressed in the neural tube, bronchi, primordial gut, early ureteric bud, mesonephric tubules, adrenal cortex, and immature hepatocytes, suggesting a role in organ development and tubular morphogenesis [12, 47]. In adult tissues, *PKHD1* is expressed predominantly in the kidney (mostly in collecting ducts and thick ascending loops of Henle), liver (localized to bile ducts), and pancreas [48, 49]. In renal tubular and biliary epithelial cells, FPC localizes to the apical membranes as well as to the primary cilia/basal body [33, 48, 50] and mitotic spindle [51].

FPC is secreted in exosomes and appears to be posttranslationally modified [52]. A subset of membrane-bound FPC undergoes Notch-like proteolytic processing, with shedding of the extracellular domain into the tubular lumen and nuclear translocation of the C-terminus, where it may play a role in downstream signaling [53–55]. However, predictions regarding the nuclear function of the cytoplasmic C-terminus are confounded by a mouse model that lacks most of the C-terminus and is phenotypically normal, without renal or hepatic disease even when aged [49].

The function of FPC is slowly coming into focus. In animal studies, mice harboring mutations in both *Pkhd1* and either of the ADPKD genes (*Pkd1* or *Pkd2*) display cystic phenotypes that are much more severe than those expressed with either mutation alone, indicating genetic interactions in vivo [56–58]. However, a direct physical interaction between FPC and the *PKD1* gene product, PC1, has not been demonstrated. Moreover, the functional significance of the reported interaction of FPC with the *PKD2* gene product, PC2, is uncertain since *Pkhd1* mutant mice lacking the FPC-PC2 interaction domain are phenotypically normal [49].

FPC appears to indirectly regulate the function of the C2-WWW-HECT domain E3 family of ubiquitin ligases by affecting the subcellular localization of NDFIP2, a protein implicated in regulating the trafficking and function of the E3 ubiquitin ligase family [59]. This protein family includes SMURFs and NEDD4, which are known to regulate the activity of RhoA, TGF-β signaling, and ENaC activity, all of which have been reported to be upregulated in ARPKD renal and biliary epithelial cells. These data may provide a unifying explanation for the observed changes in cytoskeletal structure, increased fibrosis, and enhanced sodium absorption and hypertension, but the mechanism through which FPC modulates NDFIP2 function remains unknown.

The reported localization of FPC to the basal body/primary cilium provides another intriguing, but incomplete insight into its function. As noted, FPC, DZIP1L, and other proteins associated with the hepatorenal fibrocystic diseases (e.g., ADPKD, nephronophthisis, and Meckel, Joubert, and Bardet-Biedl syndromes) also localize to these structures (Fig. 3). Taken together, these

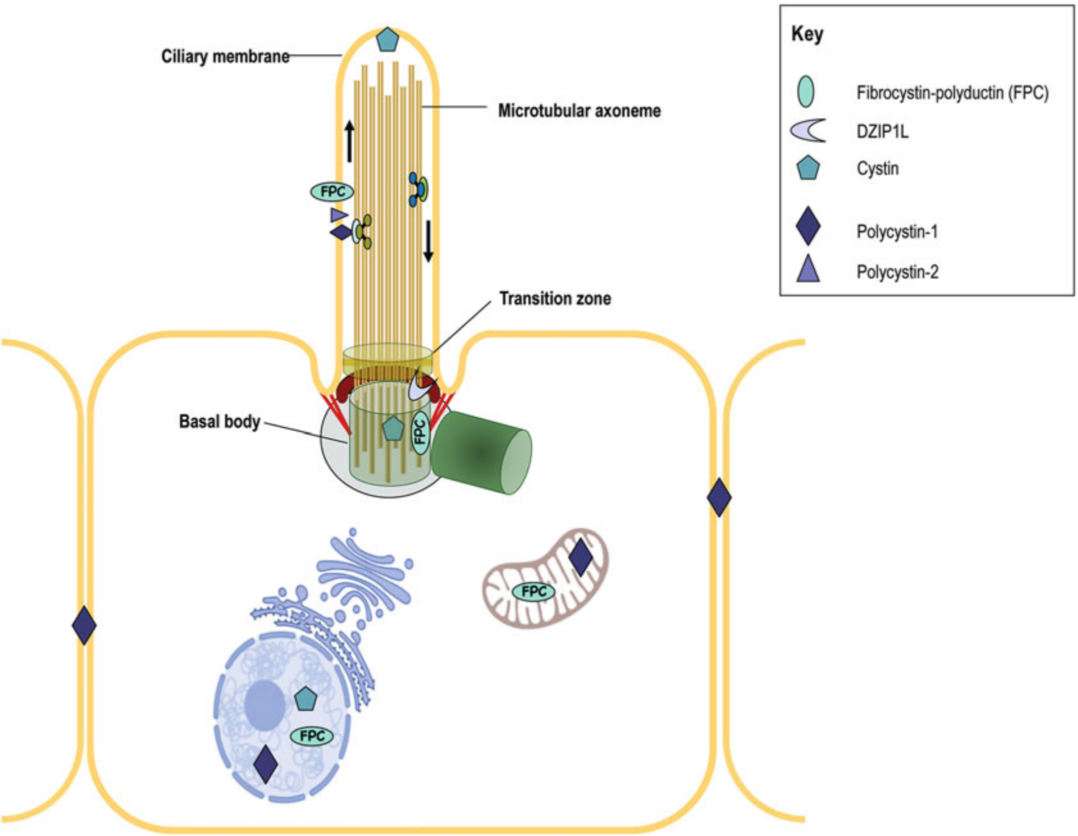

Fig. 3 Cellular localization of ARPKD-relevant proteins. FPC localizes to several subcellular compartments. These include the basal body/primary cilium, where polycystin-1, polycystin-2, cystin, and other proteins associated with the hepatorenal fibrocystic diseases also have been detected. FPC colocalizes with DZIP1L in the transition zone and cystin in the basal body. FPC and PC-1 have also been localized to the mitochondria and PC-1 to cell-cell junctions. The carboxy-terminus of FPC appears to undergo regulated translocation to the nucleus, where like cystin and PC-1, it may be involved in transcriptional regulation

data suggest a central role for the primary cilium in the development and maintenance of renal tubular architecture, perhaps via mechanisms such as flow sensing and establishment of planar cell polarity, although the specific function of FPC in any of these processes remains undefined [60].

As in ADPKD, the cAMP signaling system is activated in ARPKD (reviewed in [61]), as is the mTOR pathway [62]. In addition, new data implicate bioenergetic remodeling in both ADPKD and ARPKD pathogenesis [63, 64].

However, translating any of these observations to human clinical trials has been confounded by incongruities between human disease and experimental models as well as the lack of noninvasive markers to track disease progression and assess outcomes. For example, while agents that target cAMP activation effectively attenuated cystic disease progression in the *pck* rat (orthologous ARPKD model) [65], mTOR inhibition in this rat model failed to impact disease in either the kidney and liver disease [66]. Thus, current efforts are focused on optimizing preclinical studies with targeted therapeutics in experimental models and developing noninvasive markers for human clinical trials, which assess progression endpoints and are acceptable to regulatory agencies.

Clinical Disease Course and Clinical Management

ARPKD is typically diagnosed in the perinatal period, and despite dramatic advances in neonatal care, is still associated with significant morbidity and mortality. Current knowledge about ARPKD clinical manifestations is derived primarily from cohort studies, and thus the conclusions are greatly influenced by the way cohorts were selected. Some studies relied on recruitment from pediatric nephrology centers, while other reports were based on single-center experience. Furthermore, the genetic resolution of the disease-causing gene was not possible in earlier cohort studies or relied on linkage analysis for presumptive diagnosis. Thus, these cohorts likely included patients with phenocopy disorders due to mutations in other renal cystic disease genes. Nevertheless, there can be value in studies that relied solely on the clinical diagnosis and some cases, comparison of sub-cohorts with and without genetic findings may yield informative insights.

Most management recommendations remain opinion-based, with treatment strategies focused on ARPKD associated findings, such as hypertension [4, 5]. An 2014 international consensus report summarized management recommendations for ARPKD [4] and a more recently, a second international group has provided guidelines for perinatal management of cystic kidney diseases in general [5].

Perinatal Period

ARPKD is currently diagnosed primarily in the latter stages of pregnancy or at birth, with enlarged, hyperechogenic kidneys. As noted, distinguishing ARPKD from other phenocopy disorders can be challenging. Extrarenal manifestations can be a help to guide the clinical diagnosis [5]. Normal antenatal ultrasound findings do not exclude ARPKD, especially early in pregnancy. Oligo- or anhydramnios may develop as a result of renal functional impairment and portend worse neonatal outcomes [16]. In the presence of oligo- or anhydramnios, severely affected fetuses may develop the “Potter” phenotype with pulmonary hypoplasia, contracted limbs with club feet, and a characteristic facies. Pulmonary hypoplasia still poses a severe clinical challenge and often dictates postnatal survival [5]. Serial amnio infusions have been administered in a small number of patients, but the risk-benefit of these interventions remains to be determined. After birth, severely affected infants may require aggressive ventilatory management and pneumothoraces may become a complicating issue [5].

Current recommendations suggest that delivery of infants with suspected ARPKD should be arranged for centers with specialized neonatal and pediatric nephrology teams [4, 5]. Families of severely affected neonates may require counseling to help determine whether to proceed with aggressive care versus opt for palliative treatment. Decisions to start, withhold, or discontinue treatment often depend on a combination of patient-specific issues and family-specific factors. Multidisciplinary support and shared decision-making that is supported by a formalized ethical framework may be helpful [5].

Overall, advances in pediatric and neonatal intensive care, including sophisticated ventilation strategies, use of surfactant, improved feeding methods, as well as improved renal replacement therapy (RRT) in very young children have improved survival rates for ARPKD patients, many of whom now reach adulthood. While previous estimates suggested that up to 30% of ARPKD neonates die shortly after birth due to respiratory insufficiency [1, 30], a recent EHR-based analysis in the USA calculated a 79% perinatal survival rate [10]. Reports based on cohorts who were born in the 1990s and early 2000s have demonstrated 10-year survival rates of up to >90% for those ARPKD patients who survived the first month of life [1, 5].

Kidney Disease

Prenatal prognostic markers that predict postnatal kidney function are not well-established for

ARPKD. In the largest cohort study reported to date, involving 385 patients, enlarged kidneys, kidney cysts, and oligo-/anhydramnios were identified as sonographic predictors of early postnatal dependency on renal replacement therapy [16]. Documentation of low amniotic fluid volumes, prenatal renal enlargement, low Apgar scores, and requirement for postnatal respiratory support were independently associated with an elevated hazard ratio for dialysis dependency in the first year of life. These data may provide the basis for a predictive algorithm that identifies subgroups of patients at high-risk for an early severe kidney phenotype [16].

While decreased kidney function is common in ARPKD neonates, kidney failure rarely is a cause of neonatal death. Urine output and serum electrolytes should be monitored in neonates with significant kidney disease, e.g., those with a history of oligohydramnios [5]. Kidney function may improve during the first months of life, with subsequent progressive loss of function that can be highly variable among affected patients. A single study suggests that loss of renal function may accelerate after 10 years of age [67]. Overall, eGFR decline in ARPKD children is not substantially different from the rate of functional decline in children with chronic kidney disease (CKD) due to other underlying diseases. Perinatal clinical presentation seems to portend poorer kidney outcomes than that observed in children with later diagnosis. In one cohort, 25% of the children diagnosed in the perinatal period required kidney replacement therapy by 11 years of age, whereas for children diagnosed after the first year of life, only 25% required transplantation by 32 years of age [24].

Kidney disease management should follow the established pediatric CKD guidelines [4]. Systemic hypertension occurs in up to 80% of ARPKD patients, often developing in the first months of life [30, 31]. The precise pathogenic mechanisms are not well understood, but available data suggest that systemic hypertension in ARPKD is due to dysregulation of tubular sodium and water handling, resulting in volume overload [4]. Hypertension may improve after the first year of life, potentially in parallel to the decline in kidney function.

Antihypertension management can be very challenging and require the use of multiple drug classes. Antagonizing the renin angiotensin system with ACE inhibitors or AT1 receptor blockers is recommended as first-line therapy, based the effectiveness in the general pediatric CKD population and preclinical data indicating activation of RAS in experimental PKD models [4]. General principles of antihypertensive treatment apply as evidence remains too limited for ARPKD-specific recommendations.

Beta blockers may be considered in patients with evidence of CHF and portal hypertension. Loop diuretics could potentially lead to an upregulation of serum vasopressin levels due to the underlying urinary concentration defect and thus, could potentially trigger cyst growth. However, in many ARPKD patients, diuretics are required. Amiloride or similar agents may be considered given experimental data that show ARPKD cyst-lining cells absorb Na by a pathway that is modestly amiloride-sensitive [68].

Hyponatremia is frequently observed in ARPKD patients in the first weeks to months of life, but is often transient and resolves over time [30, 31]. Fluid overload should be avoided and care should be taken to avoid excessive sodium supplementation that might aggravate arterial hypertension [4]. Urinary tract infections are common in ARPKD children and have been reported in up to 50% of patients, with higher incidence in females [30].

ESKD has been reported in about 30% of ARPKD patients by the age of 10 years and about 40–60% of patients by the age of 20 years [31, 32]. Peritoneal dialysis and hemodialysis, as well as isolated kidney transplantation and combined liver and kidney transplantation have all been successfully used in ARPKD patients. Selecting the right modality for an individual patient depends on multiple factors, including the age of the patient, the experience of the center, and the family context. RRT is the standard approach even in neonates and young infants, although it requires an intensive personnel engagement involvement and individualized approaches. A large international cohort reported that the overall survival for RRT in neonates and infants was 81%

after 2 years [69]. In a North American study, survival was about 80% after 3 years in infants who initiated RRT in the first month of life, with 85% survival for a subgroup that initiated RRT later during the first year of life [69, 70].

The International Pediatric Peritoneal Dialysis Network (IPPN) registry systematically compared peritoneal dialysis in young children with ARPKD ($n = 79$) with two matched control groups with ESKD due either to congenital nephrotic syndrome (CNS) ($n = 79$) or congenital anomalies of the kidney and urinary tract (CAKUT) ($n = 158$) [71]. There was no significant difference in overall survival between ARPKD and CNS patients, while CAKUT patients had better overall survival. Importantly, there were no differences in the technical issues among the groups and peritoneal dialysis only required minor adaptions of prescription for ARPKD patients.

Uni- or bilateral nephrectomies are sometimes considered in infants with ARPKD, based on the assumption that massively enlarged kidneys can compromise respiratory management or nutritional support. However, the evidence base for these interventions is limited [4]. Furthermore, potential benefits need to be carefully weighed against risks and sequelae of nephrectomies. This includes issues related to unilateral nephrectomy, such as accelerated decline in renal function due to the reduction of nephron mass and rapid growth of the contralateral kidney, requiring a rapid second nephrectomy (summarized in [72]). The European ARegPKD study found that timing of nephrectomies may be an important determinant of untoward consequences [73], with bilateral nephrectomies in the first 3 months of life and hypotensive episodes emerging as independent risk factors for neurological complications later in life. Interestingly, the neurological outcome was better in a group of patients that underwent their second nephrectomy in the months 4–15 of life. These data suggest that a cautious approach is warranted when considering very early bilateral nephrectomies.

As for other causes of CKD in childhood, renal transplantation is the RRT modality of choice for ARPKD patients. Overall patient and graft survival in ARPKD patients corresponds to pediatric renal transplantation in general and this structural renal disease does not recur after transplantation. Nephrectomy of native ARPKD kidneys may be required prior to or during transplantation. Post-surgery, heightened vigilance for sepsis is warranted, as cholangitis and biliary sepsis are a major cause of mortality in ARPKD patients following isolated kidney transplantation [74, 75]. Considerations about combined liver and kidney transplantation are discussed below.

Biliary Tract Disease

As more ARPKD patients survive the perinatal period and renal disease management prolongs patient survival, liver disease is steadily becoming a major clinical issue. Liver involvement is an invariant feature of ARPKD [4, 14]. Therefore, ARPKD patients require interdisciplinary management that includes regular hepatological follow-up for progressive congenital hepatic fibrosis or Caroli's syndrome [4].

On sonography, liver parenchyma is typically inhomogeneous, the liver may be enlarged, and dilated bile ducts may be evident [17, 29, 32, 76]. Various elastography methods have been used to quantify liver fibrosis. Acoustic radiation force impulse has shown higher liver and spleen shear wave speed in ARPKD patients as compared to healthy controls, with the left liver lobe imaging having the highest sensitivity and specificity in distinguishing ARPKD subjects with portal hypertension [76]. Magnetic resonance imaging may become a useful modality to quantifying the extent of CHF [77], and provide important information on intra- and extrahepatic bile ducts, e.g., within the frame of an MRCP. Furthermore, novel magnetic resonance imaging applications are being evaluated for evaluating renal cystic disease progression [78].

Hepatocellular function is usually preserved, but progressive portal hypertension and episodes of cholangitis may complicate management [4, 29, 32]. Portal hypertension may result in splenomegaly and the development of collateral circulation. Hypersplenism may result in pancytopenia. Varices involving the esophagus, stomach, and anus may develop. Bleeding, particularly from esophageal varices, may be life-threatening and regular endoscopic evaluation including sclerotherapy or variceal banding may be required [4]. Transjugular intrahepatic portosystemic shunting (TIPS) has been successfully applied in some patients [79]. However, for patients with significant CKD, the risk of developing hyperammonemia after TIPS must be considered.

Laboratory evaluation typically reveals no biochemical evidence for hepatic dysfunction [4]. Serum liver transaminases are rarely elevated, while biliary markers, e.g., *gamma-glutamyl* transferase (GGT), can be markedly elevated. Thrombocytopenia was the best predictor of portal hypertension and splenomegaly in a cohort of 73 patients [29]. Among young adults with ARPKD, a high number of patients had different types of hepatic involvement with hepatosplenomegaly, thrombocytopenia, and inhomogeneous liver parenchyma on ultrasound being the most common findings [32]. Liver-associated issues gradually progress over the course of childhood and can represent a significant management issue in transitioning care to adult health systems.

Cholangitis is a potentially severe complication of ARPKD-related liver disease and diagnosis can be challenging. Therefore, unexplained fever in a child with ARPKD should prompt aggressive evaluation for cholangitis, particularly in patients following isolated kidney transplantation. Studies should include GGT, inflammatory markers, and cultures of blood and urine. Liver ultrasound examination may be useful. In cases of confirmed cholangitis, aggressive antimicrobial treatment is indicated. In general, antibiotic prophylaxis is not recommended [4].

In patients with CHF from a variety of causes, there is an increased risk of hepatobiliary carcinoma. This risk should be considered in the management of adult ARPKD patients, but is a very rare complication in children and young adults with ARPKD [4, 80, 81].

Transplantation

ARPKD is one of the two major pediatric indications for combined liver and kidney transplantation when a patient has marked CKD and substantial liver involvement, due to severe portal hypertension or recurrent cholangitis [82–85]. One report has described better kidney graft function after combined kidney and liver transplantation from the same donor, which would permit a less intensive immunosuppression regimen [86]. These factors must be weighed against the increased risk of combined organ transplantation and the limited number of centers that are equipped to undertake this aggressive intervention. For example, recent analysis of ARPKD patients in the ESPN transplant registry identified a 6.7-fold increased age- and sex-adjusted mortality risk over a 5-year follow-up for those undergoing combined liver and kidney transplantation ($n = 32$) compared to isolated kidney transplantation ($n = 163$) [82]. This retrospective analysis revealed that some patients undergoing combined transplantation had limited evidence of clinically significant liver disease and there was no detectable benefit for renal graft survival in this cohort. These data demonstrate the importance of careful individual decision-making in these cases. Various algorithms to guide decision-making regarding combined transplantation in ARPKD patients have been proposed but more data are needed [82–85].

Additional Features

In addition to neonatal respiratory insufficiency and fibrocystic liver disease, additional extrarenal disease manifestations must be considered in the management of ARPKD patients. Failure to thrive is a general concern in children with early-onset CKD. In ARPKD, enlarged kidneys, uremia, and respiratory challenges due to pulmonary hypoplasia may impact feeding and adequate nutrition. There is debate about whether growth delay is a primary feature of ARPKD, with two large registries finding growth delays in up to 25% of patients, whereas analysis of 22 ARPKD patients in the CKiD cohort did not identify a disease-specific effect of ARPKD on growth [31, 87, 88].

While gastrostomy tubes are often placed in children with CKD to facilitate adequate nutritional intake, in ARPKD patients, there are specific concerns about secondary splenic injury or the increased risk of bleeding due to portal hypertension [89]. A recent European ARegPKD Consortium survey of clinicians managing ARPKD patients found that most centers supported gastrostomy tube placement if clinically indicated. The reported complications were generally comparable to those in non-ARPKD patients undergoing the same procedure [90].

In ADPKD patients, the most feared extrarenal complication is the development of intracranial aneurysms. For ARPKD patients, the data are very limited [4]. While there are a growing number of case reports on vascular aneurysms (intra- and extracranial) in ARPKD patients, these appear to be rare complications of a rare disease, particularly for children and young adults [91, 92]. A specific screening protocol has not been recommended, but a low threshold for corresponding imaging seems justified.

The toll of CKD, arterial hypertension or hypotension (e.g., after nephrectomies), liver disease, and lung disease, on neurocognitive development in ARPKD was evaluated in a recent CKiD study. Intellectual functioning, academic achievement, attention regulation, executive functioning, and behavior were comparatively evaluated in 22 ARPKD children versus 44 children with other underlying causes of mild-to-moderate CKD. Children were matched for glomerular filtration rate, age at study entry, and age at diagnosis. There was no evidence for neurocognitive dysfunction in ARPKD patients [93].

Finally, as a chronic disease that typically manifests early in life and progresses through childhood, ARPKD can be challenging for both the impacted child and the family. Clinical management needs to take into consideration these psychological issues and the need for psychosocial support cannot be overstated. In addition to the care team, support is available from patient advocacy groups, e.g., PKD International in Europe or the PKD Foundations in the USA and Canada.

Future Horizon

ARPKD is a multifaceted disorder which requires expert, multidisciplinary management. Much has been learned over the past few decades about the genetics and pathobiology of this disorder. Patient survival has improved remarkably due to advances in supportive therapy, dialysis, and transplantation. The recent development of harmonized international ARPKD registries [10, 94], next-generation sequencing platforms, and new imaging modalities are laying the foundational framework for predicting postnatal outcomes, characterizing patient subsets, clarifying how genetic variants impact disease expression, and devising new strategies to monitor disease progression. In addition, there is increasing recognition that mechanistic pathways in ARPKD in large part overlap with ADPKD. Taken together, the near horizon holds the promise of developing predictive/prognostic markers and initiating clinical trials with targeted therapeutics. The ultimate goal is to identify disease-specific therapies that mitigate the inexorable progression of ARPKD-related morbidities.

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Renal Involvement in Tuberous Sclerosis Complex

John J. Bissler and Dinah Batchelor

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Abstract

Tuberous sclerosis complex (TSC) affects every organ system. Throughout the patient's lifetime, the renal manifestations become more serious. While renal disease can be life threatening in the adult with TSC, the opportunity to modify the disease course is during the pediatric time frame. Recent advances in cell biology have translated into targeted therapy for TSC renal disease and have revealed novel insights about the renal response to disease. Proper surveillance and treatment can significantly reduce the renal disease burden and prolong the duration and increase the quality for the patient.

Keywords

Tuberous sclerosis complex · Angiomyolipoma · Renal cystic disease · Oncocytoma · mTORC1 · Rapamycin · Sirolimus · Everolimus

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Introduction

Tuberous sclerosis complex (TSC) is a genetic disorder characterized by abnormal development and post-natal cellular proliferation in many parts of the body. The resulting tumors commonly occur in the skin, brain, kidneys, lungs and other organs, and in some cases leading to significant health problems. Virtually all affected people have skin abnormalities, including hypopigmented macules, subungual fibromas, and a Shagreen patch. The majority of TSC patients also have brain involvement that can lead to neuropsychiatric disorders including anxiety, depression, hyperactivity, aggression, psychiatric conditions, intellectual disability, and autism spectrum disorder. Mostly in childhood and young adulthood, patients can develop a subependymal giant cell astrocytoma (SEGA), and the incidence greatly drops off after the age of 36 years. The frequency of diseases also increases with age in some organ systems. In TSC, the prevalence of LAM increases with age reaching up to 80% in women aged 40 years [26]. Renal disease in TSC likewise increases with age. Recognition of the remarkably varied disease manifestations and severity is confounded by the rarity, interfering with accurate diagnosis and treatment of affected patients. Over 80% of affected children and all adult postmortem samples [69] were found to have renal disease, most commonly renal angiomyolipomata and cysts. Premature decline of glomerular filtration rate (GFR) occurs in ~40% of patients with TSC [10], and can occur in the absence of angiomyolipomata bleeding or interventions. TSC associated renal disease was the leading cause of death in those clinic populations [51, 66].

Genetics

Tuberous sclerosis complex, an autosomal dominant genetic disorder affecting every organ system, exhibits a birth incidence of ~1:5800 and affects approximately one million patients worldwide [24, 77]. Proper diagnosis often will be missed if one uses the outdated Vogt's triad for TSC (facial angiofibromas, developmental delay, and intractable epilepsy) because less than 40% of affected patients exhibit these classical features [48]. Only about half of patients with TSC exhibit cognitive impairment, autism, or other behavioral disorders [48]. There are two loci associated with TSC, the *TSC1* gene on chromosome 9 and the *TSC2* gene on chromosome 16. The identification of the *TSC2* gene location was facilitated by an astute genetic observation in a family with autosomal dominant polycystic kidney disease (ADPKD). The polycystic disease was caused by a balanced translocation involving the *PKD1* gene. The child in the family who had both ADPKD and tuberous sclerosis complex inherited the unbalanced translocation, the mapping of which aided in the positional cloning of the TSC2 gene [35].

Tuberous sclerosis complex generally is thought to be phenotypically expressed by a second somatic silencing event of the non-mutant allele, either through a second somatic mutation or other possibly silencing mechanisms. Both TSC and autosomal dominant polycystic kidney disease are phenotypically expressed due to a second-hit, or somatic mutation mechanism [21, 43]. The kidney disease associated with chromosome 16, specifically the *PKD1* and the *TSC2* loci, account for a majority of their respective diseases, and exhibit a more severe phenotype compared to the disease associated with the other loci (*PKD2* and *TSC1*). This association of the short arm of chromosome 16 with more severe disease appears to have a molecular underpinning. The *PKD1* and *TSC2* genes are adjacent, in a tail-to-tail orientation, and the *PKD1* gene contains an intronic sequence that has unique structural properties [19] that can predispose to mutation. This odd *PKD1* sequence interferes with DNA replication and causes DNA double-strand breaks resulting in mutations [50, 61]. Synergizing with this predisposition to DNA double-strand breaks, the renal microenvironment also inhibits recognition of DNA damage [30–32].

Clinical Aspects and Treatment of TSC Renal Disease

General Clinical Aspects

Multidisciplinary Care

Due to the many manifestations of TSC disease and the serious complications, management requires the expertise of multiple medical disciplines [4]. To adequately care for patients, consensus guidelines recommend a multidisciplinary care approach [49]. The use of multidisciplinary teams is beneficial in treating other complex diseases, such as cancer. The care of the renal patient really relies on experience.

Chronic Kidney Disease

By their mid 50s, 41% of patients with TSC have exhibited stage 3 or greater chronic kidney disease (CKD), compared to 3% of the general population [10, 46]. Although, the risk of end stage renal failure in which families opt for renal replacement therapy appears to be about ten-fold lower [34]. Therefore, TSC patients with renal disease are at an increased risk for premature cardiovascular disease [56] and may succumb before they reach end stage renal failure. There are multiple potential causes for this premature loss of GFR, including acute kidney injury from renal hemorrhage, loss of normal renal parenchyma following embolization or surgery, hypertension, and replacement of normal renal cells with angiomyolipomata or cysts. Also, it has been suggested that damage is caused to normal renal tissue by multiple angiomyolipomata. GFR can be normal even when there is no recognizable renal parenchyma on imaging. Impaired kidney function has been identified more commonly in patients who had undergone embolization (29%) than in those who had not (10%). However, this finding may represent a selection bias because of the high burden of angiomyolipomata in those who subsequently needed embolization [62]. Hopefully mTORC1 inhibitor therapy will ameliorate renal angiomyolipomata and will improve long-term outcomes of renal function. Preliminary evidence of a very low complication rate—including low levels of renal impairment—in the TOSCA cohort support that this is a useful strategy [48].

Hypertension

Hypertension is more common in patients with renal TSC than in the general population [44]. Hypertension is a known risk factor for progression of renal scarring and accelerates GFR loss in patients with renal disease if not treated appropriately. Standard recommended blood pressure targets for adult TSC patients is ≤130/80 and appropriate 70% for age adjusted targets for children [36]. There is concern about using angiotensin converting enzyme inhibitors (ACEIs) in those TSC patients taking mTORC1 inhibitors based on a possible increase incidence of angioedema in kidney transplant patients taking both [33]. However, ACEIs and angiotensin receptor blockers are extremely useful in this population and may suppress the growth of angiomyolipomata [68] and cysts [63]. Angioedema due to the combined administration of mTORC1 inhibitors and angiotensin converting enzyme inhibitors has not been reported in the TSC population as a drug limiting problem, and they can be used concurrently with caution [28].

Specific Aspects Including Diagnosis, Surveillance, and Treatment of TSC Associated Renal Disease

There are specific aspects of TSC renal care that deserve attention. These include the renal angiomyolipomata and cystic disease. While the hemorrhage risk of angiomyolipomata and the need for treatment is becoming better understood, the health risk of TSC renal cystic disease is still less appreciated.

Angiomyolipomata

Angiomyolipomata detection in patients with TSC increases from 8.8% in patients less than 2 years of age to 78.9% in those patients greater than 40 years of age [11]. Likewise, the number of patients requiring intervention also rises from 1% in the under 2 year-old up to 48.6% in those over 40 [48].

However, the imaging modality is a source of confusion. About a third of TSC-associated angiomyolipomata seen in a TSC Center of Excellence with expertise in TSC Nephrology have significant fat-poor components. Because these fat-poor lesions have similar tissue density as renal parenchyma, they have echotexture similar to kidney and are often not identified using ultrasound. This reason is part of the rationale behind the International Consensus Guidelines that recommend MRI as screening for TSC patients [49, 58]. Because at least half of the patients affected with TSC will have cystic disease on MRI, it is common for some patients to have a solid mass that is in close proximity to cystic components. In the general population, such findings raise concern for renal cell carcinoma. They should not raise the same level of concern in the TSC patient population because fat poor angiomyolipomata are much more common than the rare renal cell carcinoma. Such lesions can safely be serially assessed for growth to help distinguish fat-poor angiomyolipoma from malignancy [62].

Lesion hemorrhage is a potential complication of angiomyolipomata which had historically been reported to occur in 25–50% of patients [45, 57]. More recently, it has been reported to occur in about 30% of patients in a larger population-based study of patients not having active surveillance [47]. One explanation for this is the propensity for aneurysm formation which involves the vascular pericytes and their larger vessel equivalent, the adventitial cells. Given their likely role in the renal disease [68], it follows that such cells, with a loss of TSC gene function, would be compromised in their normal function of choreographing vessel structure and function, facilitating aneurysm formation. Aneurysms larger than 5 millimeters are at higher risk of rupturing [74]. They are best approached using arterial embolization plus a corticosteroid taper over a week or two to reduce the post-embolization syndrome [11]. Angiomyolipomata are detectable at a younger mean age in patients with *TSC2* mutations compared to *TSC1* (13 years vs. 24 years) [48]. Intervention is required more often in patients with a *TSC2* mutation compared to *TSC1* (27% vs. 13%) and have equal prevalence between males and females with TSC. The risk of aneurysms rupturing, resulting in hemorrhage is also associated with lesion size; those 30 mm in diameter and still enlarging are at the greatest risk.

The recommendations for treatment have radically been altered because of discoveries in cell biology leading to clinical trials. A decade ago, recommendations centered on embolization and, if required, urologic surgery. Now, the International Guidelines for surveillance and management of TSC recommend preemptive treatment of enlarging angiomyolipomata that are >30 mm in diameter with an mTORC1 inhibitor to prevent hemorrhage [49]. The soundness of this recommendation can be appreciated by considering that a proactive experience of monitoring and preemptive embolization did improve the high risk of bleeding, but there was still significant renal morbidity and mortality. Eijkemans et al. reported 57 patients that required two or more embolization procedures, 16 requiring nephrectomy, 7 dialysis, and 7 transplantation, and that of the 29 deaths, 9 were due to renal causes [34]. With the advent of mTOR inhibitors replacing embolization as first choice for preemptive therapy, complications such as hemorrhage have been reduced to just 5% of 2216 subjects in one large study [48]. A series of phases 2 and 3 studies has shown that mTORC1 inhibitor therapy is highly effective at stabilizing or shrinking angiomyolipomata in both adults [12, 13, 27] and children [14] in the short term, and preventing bleeding and preserving renal function in both groups in the long term [16].

In the setting of TSC, angiomyolipomata are classified as benign lesions, though non-TSC varieties can be aggressive [2], and there are rare reports of TSC angiomyolipomata exhibiting malignant features [1]. In the non-TSC patients, the malignant angiomyolipomata are associated with mutations in MITF transcription family genes that are known to have a significant link to malignancy [29]. Although angiomyolipomata are often begin in childhood TSC patients, the majority continue to grow life-long. Most commonly, they involve the kidney but also the liver, local lymphatics or elsewhere, often in the abdomen.

Table 1 **Sages of angiomyolipomata and cystic disease.** Angiomyolipomata stages are defined by the number and size of the lesions as well as the renal morphology. This latter component helps catch the infiltrative lesions. Cystic disease is described by the number of cysts or degree of renal involvement

Stage	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Angiomyolipomata							
Number	None	1 to 4	>5	All <5	>5	>5	>5
Size	*None*	*<3 cm*	*<3*	*1 > 3 cm*	*2 to 4 are >3 cm*	*>5 are >3 cm*	*1 > 5 cm*
Morphology	*Normal*	*Normal*	*Normal*	*Intact*	*Intact*	*Recognizable*	*Nonrecognizable*
Cystic disease							
Polycystic	0	<2	3–6	7–10	11–20	21–30	>31
Cortical cystic	0	1–10	11–20	21–30	31–40	41–50	>51
Multicystic	0	1–10	11–20	21–30	31–40	41–50	>51
Focal cystic	0	1 ray	2 ray	3 ray	4 ray	5 ray	>5 ray
Microcystic	0	10%	20%	30%	40%	50%	>50%

Guidelines for surveillance and treatment of angiomyolipoma in patients with TSC are straight forward. In order to avoid failing to detect fat poor lesions, MRI is recommended for screening and monitoring renal angiomyolipomata at diagnosis and every 1–3 years following diagnosis [58]. A clinical system of scoring for angiomyolipomata (Table 1) is useful, especially given the regression induced by mTORC1 inhibitor therapy [15]. Other aspects of TSC associated disease are also responsive to mTORC1 inhibition. The brain lesion, subependymal giant cell astrocytoma (SEGA), that occurs in about 20% of patients with TSC respond to mTORC1 inhibitors [37], as does the lung disease, lymphangioleiomyomatosis (LAM) [12, 55].

Cystic Disease

TSC renal cystic disease is identified by conventional MRI in approximately 50% of patients. The size of the renal cysts in TSC renal disease range from the microscopic glomerulocystic to a polycystic renal phenotype associated with the TSC2/PKD1 contiguous gene syndrome. There are five basic patterns of cystic disease described (Table 1, Fig. 1), and there seems to be a cortical/column of Bertin predilection early on in the disease process. While there are no approved treatments of TSC associated renal cystic disease, blood pressure control using angiotensin converting enzyme inhibitors or angiotensin receptor blockers has been adopted, as this was effective in the HALT trial that has tested these drugs in ADPKD [64]. Small cystic lesions can also be mTORC1 responsive [17]. In order to communicate the actual cystic disease, the disease patterns are described below.

TSC Polycystic Kidney Disease

This manifestation, accounting for about 2% of TSC renal disease, is most often associated with a deletion involving the adjacent *TSC2* and *PKD1* genes on chromosome 16p13, and there is a high frequency of mosaicism [23]. Hypertension is often discovered by the second week of life but can take several months to develop, and sometimes it does not develop. Because of significant renal parenchyma disruption, these children sometimes develop a urinary concentrating defect very early. The lining of these cysts appears to be intercalated cells, and may arise from a mechanism distinct, at least in part, from ADPKD [17]. Because the kidney mass can be so great, learning to walk as a developmental milestone may be delayed purely due to mechanical issues. Nocturnal enuresis is often prolonged because of the concentration defect, and ddAVP should be avoided given the unknown effect it may exert on cyst growth. If blood pressure is well-controlled, patients often maintain renal function until young adults. mTORC1 inhibition in this form of cystic disease will only affect the small cysts and have no effect on the large ones.

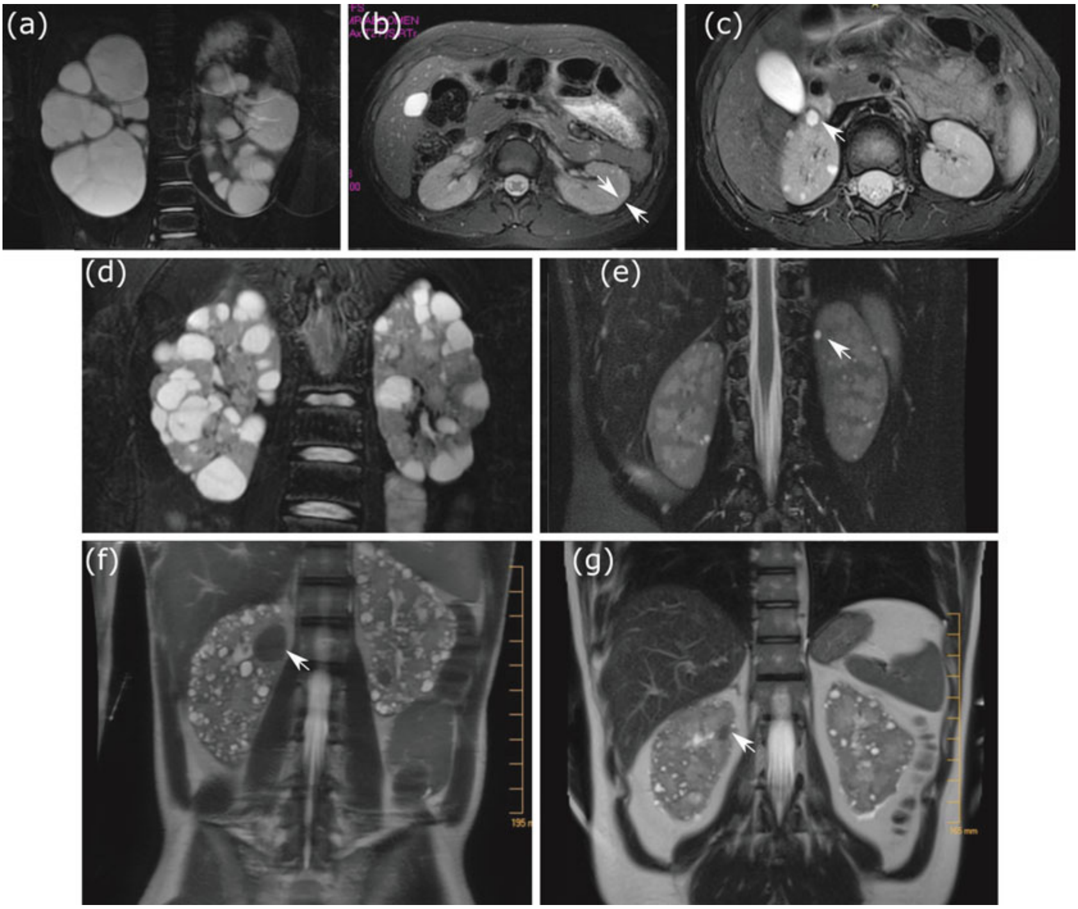

Fig. 1 Phenotypes of TSC renal cystic disease. (**a**) Polycystic variety. The white cysts are easily seen in the T2 fat suppressed image (**b**) Microcystic disease. Note, the cortex is too 'white' and there are some cortical cysts visible (white arrows to demonstrate whiteness of cortex). (**c**) Focal cystic disease. White arrow denotes eight separate cysts in a cluster. (**d**) Multicystic disease in a patient with *TSC1* mutation (**e**) Cortical cystic disease with cysts limited to cortex and columns of Bertin. White arrow denotes a single cyst. (**f**) Typical patient with both angiomyolipoma (white arrow) and stage 6 cortical cystic disease. (**g**) Same patient as in figure 'f' after 3 years of mTORC1 inhibition. Note angiomyolipoma shrinkage (white arrow) and significant improvement in cystic disease. (Figure (**b**) used with permission from [15])

TSC Microcystic Kidney Disease

The MRI findings of microcystic disease are subtle and are most often missed on ultrasound. The renal cortex exhibits increased water signal on T2 sequences, preceding discrete cortical cysts. While easily overlooked, this pattern of TSC-associated renal cystic disease is important because it is associated with a rapid decline in renal function, with patients developing chronic kidney disease stage 2 or 3 in their late teens or early 20s. Renal pyramids are spared so the urinary concentration capacity is preserved. There can be evidence of tubular proteinuria with increasing creatinine, but hypertension is not common until significant chronic kidney disease develops. This pattern does seem to be mTORC1 inhibitor responsive, though more research is needed.

TSC Focal Cystic Kidney Disease

Focal cystic disease manifests as a cluster of cysts and may be the result of a somatic mutation during branching morphogenesis, so that there is significant localized cystogenesis. The developmental timing can be in utero, resulting in an infant with an isolated site with profound cystic disease. The mutation may occur after a critical developmental period, and only become expressed after renal healing following acute kidney injury.

TSC Cortical Cystic Kidney Disease

A common distinct pattern of TSC cystic renal disease features cysts limited to the cortex and columns of Bertin. This cystic disease occurs early and the cysts initially exhibit a remarkably uniform size. This imaging pattern may suggest dilatation of tubular segments, or perhaps glomerulocystic disease.

TSC Multicystic Kidney Disease

The cysts in this pattern can be distributed throughout the cortical and medullary tissue and exhibit variable sizes with loss of corticomedullary differentiation. This pattern can be severe enough that it resembles the polycystic variety but is genetically distinct from the *TSC2/PKD1* contiguous gene syndrome.

Practical Approach to Surveillance and Treatment

Care of the TSC patient with renal involvement is best approached in a partnership with a TSC Center of Excellence. The newly diagnosed or suspected patient requires an abdominal MRI to assess for renal cysts and angiomyolipomata. MRI is required. Because of the high rate of fat poor lesions that have the same echotexture as normal kidney, making them virtually invisible to ultrasound. These patients also required renal function testing and blood pressure assessment. Patients with known disease require abdominal MRI every 1-to-3 years along with annual blood pressure and renal function testing. Asymptomatic, growing angiomyolipoma greater than three centimeters in diameter should be treated with an mTORC1 inhibitor, though a second-line approach for exophytic lesions could be sub-selective embolization or renal sparing surgery. Hemorrhaging angiomyolipomata should be treated with sub-selective embolization and the use of a corticosteroid protocol can eliminate the post-embolization syndrome.

Cell Biology of TSC Renal Disease

The basic science underpinning of TSC renal disease, in many ways, gave rise to pharmacotherapy. Understanding the cell biology of the disease will illuminate additional therapeutic avenues.

Angiomyolipomata

The cell of origin for the angiomyolipomata, categorized as a perivascular epithelial cell tumor (PEComa), is unclear. A neural crest cell of origin was suggested based on co-expression of melanocytic and smooth muscle cell markers [25, 40, 42]. However, re-expression of *TSC2* in an angiomyolipoma cell line altered the expression profile leading the investigators to doubt the neural crest cell origin and favor a lymphatic endothelium origin [78]. A dual knock-out of *Tsc2* and *Cdkn2a* in murine epithelial cells altered the cell expression to be somewhat similar to angiomyolipoma. However, *Cdkn2a* mutations are associated with melanoma, and this may confound the possible significance of the human melanoma black (HMB)-45 staining. The HMB-45 antibody is directed at premelanosome protein (PMEL)-17 and is useful in staining melanoma, angiomyolipoma, and the MITF transcription factor renal tumors. Mesenchymal stem cell-like cells have been identified in angiomyolipomata [75], and pericytes have been implicated as the cell of origin for angiomyolipomata [68]. This latter possibility is intriguing given that vascular aneurysms associated with TSC can arise in angiomyolipomata [74], the aorta [66], and the brain [7].

Angiomyolipomata belong to the PEComa family of tumors. This family is a unique and diverse group of tumors that exhibit both smooth muscle and melanocytic differentiation characteristics [59, 71]. Angiomyolipomas often have a triphasic appearance that includes thick-walled blood vessels, myoid-appearing perivascular cells, and lipid-distended cells resembling adipocytes. They can also resemble epithelioid cells and can have a very different mix of cells from tumor to tumor. There is an interesting interplay between the microphthalmia-associated transcription factor (MITF) family and the mTORC1 pathway, as is clearly suggested by the fact that angiomyolipomata express the melanotic marker PMEL (premelanosome protein, also known as SILV, gp100), the antigenic target for the diagnostic antibody HMB45 [76]. An interplay is also suggested by the hypopigmented macules that are part of the major diagnostic criteria [80]. This MITF/TFE transcription factor activity has also contributed to confusion between TSC-associated renal lesions and the much more aggressive renal cell carcinomas (RCCs) and PEComas associated with translocations involving TFE3 or TFEB [2].

Cystic Disease

The mTORC1 pathway is associated with primary cilia-associated cystogenesis. Unilateral nephrectomy in transgenic mice that underwent "adult-induced" cilia disruption exhibited significantly higher levels of mTORC1 activity in the remaining kidney compared to cilia-intact controls with increased cyst severity [6]. Although the polycystic disease-related proteins and primary cilia are mechanistically involved in polycystic kidney disease, they are not sufficient as the mediator. The loss of a polycystin protein or the primary cilia result in similar renal cystic disease phenotypes, but simultaneous inactivation of both the polycystin and cilia resulted in a decrease in PKD severity compared with polycystin-only knockout [52]. The polycystins must modulate an unidentified signaling pathway that requires intact cilia to function, implicating the primary cilia-extracellular vesicle axis. Extracellular vesicles (EVs) carry cargo including modulators of polycystic disease signaling, such as Hedgehog [67] and Wnt [65].

To understand the cystic disease in TSC better, a novel mouse model that inactivated the *Tsc2* gene in principal cells was generated by breeding a floxed *Tsc2* mouse to one containing Cre-recombinase driven by the aquaporin-2 promoter. The resulting mice have normal kidneys at birth but develop florid cystic disease by 19 weeks of age. Curiously, although the *Tsc2* gene was deleted in principal cells, the cysts were comprised of type A intercalated cells that contained an intact *Tsc2* gene [17]. Type A intercalated cells were also identified to be involved in human TSC-related cystic disease [17]. The mechanism for this cell nonautonomous trait may utilize EVs to reprogram cells [79], as has been suggested for another tumor predisposition syndrome. Chodankar et al. used a similar Cre-lox system to inactivate *Brca1* in mouse ovarian granulosa cells by using a truncated form of the *Fsh* receptor promoter to drive Cre recombinase. The authors demonstrated that inactivation of the *Brca1* gene in granulosa cells led to the development of cystic tumors with genetically intact *Brca1* genes in the ovaries and uterine horns. They posited that the loss of *Brca1* may influence tumor development indirectly through some form of effector secreted by granulosa cells [22].

In mouse models studied, there seems to be significant elevation of cyst mTORC1 activity (phospho-S6 expression), but a very low percentage of cells exhibiting loss of Tsc expression [3, 60, 81]. The somatic mutation (second hit) dogma of TSC renal disease is difficult to reconcile with the early investigations that failed to find loss of heterozygosity in a majority of cysts, indicating that the majority of renal cysts maintain their *Tsc* locus integrity [60, 72]. This low percentage of cysts with loss of heterozygosity is similar to human TSC renal cystic disease, because human cysts also continue to express tuberin and hamartin. In contrast, the mechanism for angiomyolipomata shows an inactivating mutation and loss of heterozygosity resulting in the loss of tuberin staining [20]. Such a low percentage of loss of heterozygosity is also seen in PKD1 associated autosomal dominant polycystic kidney disease, suggesting that such cystic disease may represent a unique disease mechanism [5, 21].

Oncocytoma and Renal Cell Carcinoma

Patients with TSC are also reported to have renal oncocytomas and renal cell carcinomas [8]. Renal oncocytomas histologically contain nests and tubular structures lined by cells with eosinophilic, granular cytoplasm [73]. Histologic diagnosis is challenging when lesion patterns are complex. For example, a solid lesion with highly compact nested architecture or with small papillary structures protruding into cystic spaces is similar to the eosinophilic variant of papillary renal cell carcinoma. Oncocytomas contain edematous myxoid or hyalinized stroma with some areas with nests and tubular structures. Clear cytoplasm may be focally present, and cells in these lesions have round nuclei and can contain areas of degenerative cytologic atypia. These features result in patches of tumor cells containing large nuclei, irregular nuclear contours, and smudged chromatin [70]. This lesion is almost always described as benign. Renal cell carcinoma is also described in patients with TSC [18], but the frequency for TSC is likely much lower and inflated estimates of 2% are difficult to substantiate because the data are rarely in collected series [41]. In the largest retrospective study of 2216 subjects, renal malignancy was reported in 1.1% of subjects [48]. Other inherited renal cancer predisposition syndromes have a more elevated risk, such as Birt-Hogg-Dubé with an estimated frequency for renal malignancy of about 25% [53].

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Nephronophthisis and Related Ciliopathies

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Daniela A. Braun and Friedhelm Hildebrandt

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Abstract

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease that constitutes one of the most frequent genetic causes for end-stage kidney disease (ESKD) in the first three decades of life [1–4]. Three clinical forms of NPHP have been distinguished by age of onset of ESKD: infantile [5, 6], juvenile [7], and adolescent NPHP [8], which manifest with ESKD at median ages of 1 year, 13 years, and 19 years, respectively. Initial symptoms are relatively mild with the exception of infantile NPHP type 2. They consist of polyuria, polydipsia with regular fluid intake at nighttime, secondary enuresis, anemia, and growth retardation [8]. A slightly raised serum creatinine is noted at an average age of 9 years, before ESKD invariably develops within a few years (Fig. 1a). Renal ultrasound reveals increased

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echogenicity (Fig. 1b). Beyond the age of 9 years, cysts appear at the corticomedullary junction within the kidneys of normal or slightly reduced size (Fig. 1b) [9]. Renal histology reveals a characteristic triad of tubular basement membrane disruption, tubulointerstitial nephropathy, and cysts (Fig. 1c) [10, 11]. In nephronophthisis, cysts arise from the corticomedullary junction of the kidneys (Fig. 1b). Because kidney size is normal or slightly reduced, cysts seem to develop ex vacuo through loss of normal tissue. This is in contrast to polycystic kidney disease, where cysts are distributed evenly and lead to gross enlargement of the kidneys [12]. NPHP is part of a broad spectrum of renal cystic/degenerative diseases that often include extrarenal manifestations. Over 90 recessive single-gene causes have been identified. Because the related gene products localize to primary cilia and centrosomes, the term “NPHP-related ciliopathies (NPHP-RC)” is now used for these disorders.

Keywords

Nephronophthisis · Ciliopathies · Pediatric renal-cystic disease · Inherited kidney diseases · Tubulo-interstitial renal fibrosis · Molecular genetic diagnostics

Synonyms

Nephronophthisis – NPHP; NPHP-related ciliopathies – NPHP-RC – renal ciliopathies

Natural History of Nephronophthisis and Related Ciliopathies

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease that constitutes one of the most frequent genetic causes for end-stage kidney disease (ESKD) in the first three decades of life [1–4]. Three clinical forms of NPHP have been distinguished by age of onset of ESKD: infantile [5, 6], juvenile [7], and adolescent NPHP [8], which manifest with ESKD at median ages of 1 year, 13 years, and 19 years, respectively. Initial symptoms are relatively mild with the exception of infantile NPHP type 2. They consist of polyuria, polydipsia with regular fluid intake at nighttime, secondary enuresis, anemia, and growth retardation [8]. A slightly raised serum creatinine is noted at an average age of 9 years, before ESKD invariably develops within a few years (Fig. 1). Renal ultrasound reveals increased echogenicity (Fig. 1b). Beyond the age of 9 years, cysts appear at the corticomedullary junction within the kidneys of normal or slightly reduced size (Fig. 1b) [9]. Renal histology reveals a characteristic triad of tubular basement membrane disruption, tubulointerstitial nephropathy, and cysts (Fig. 1c) [10, 11]. In nephronophthisis, cysts arise from the corticomedullary junction of the kidneys (Fig. 1b). Because kidney size is normal or slightly reduced, cysts seem to develop ex vacuo through loss of normal tissue. This is in contrast to polycystic kidney disease, where cysts are distributed evenly and lead to gross enlargement of the kidneys [12]. NPHP is part of a broad spectrum of renal cystic/degenerative diseases that often include extrarenal manifestations. Over 90 recessive single-gene causes have been identified. Because the related gene products localize to primary cilia and centrosomes, the term “NPHP-related ciliopathies (NPHP-RC)” is now used for these disorders.

NPHP-RC are inherited in an autosomal recessive mode and include NPHP-RC variants with extrarenal manifestations [1, 12, 14], which are present in more than 20% of patients with NPHP. Extrarenal involvement primarily includes retinal degeneration (Senior-Loken syndrome) [15, 16], cerebellar vermis aplasia (Joubert syndrome) [17, 18], liver fibrosis [19, 20], cone-shaped epiphyses [21, 22], and thoracic dystrophy (Jeune syndrome) [22]. The most severe syndromic manifestation of ciliopathies, Meckel-Gruber syndrome, is characterized by severe dysplastic multi-organ defects and embryonic lethality [23]. Extrarenal manifestations of NPHP-RC will be discussed below in light of the cilia/centrosome theory. NPHP has previously been grouped together with the clinical entity of

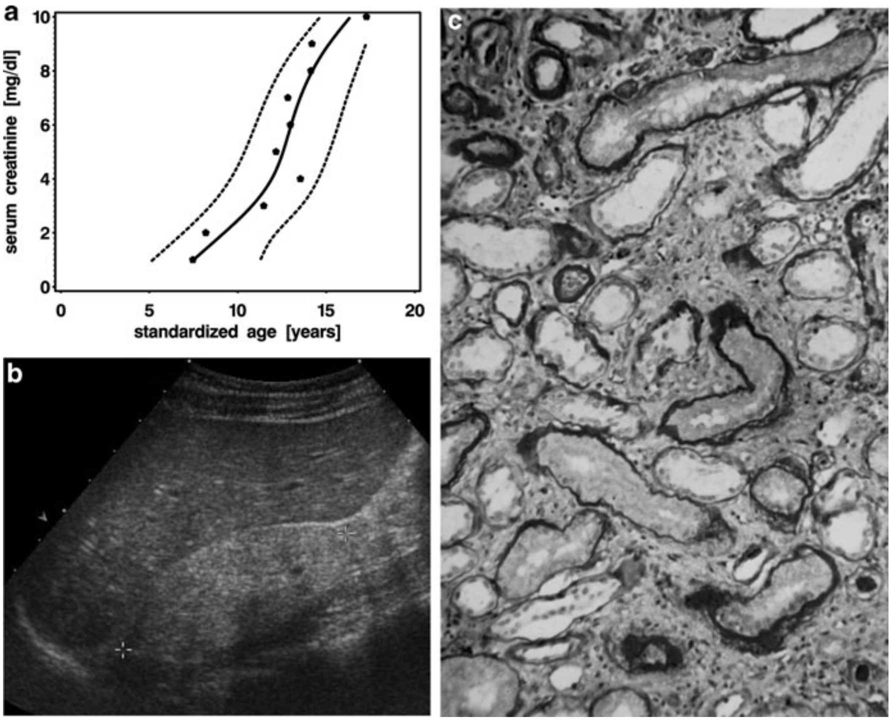

Fig. 1 (**a**) Progression chart representing the average course of deterioration of renal function in 19 patients of eight families with NPHP type 1 as proven by homozygous deletion of the *NPHP1* gene. Median (solid line) and quartile (dashed lines) curves were calculated from 308 serial SCR values (Reproduced with permission from [13]). (**b**) Renal ultrasound in nephronophthisis. Note the kidney of normal size (12 cm between markings), loss of corticomedullary differentiation, presence of cysts at the corticomedullary border of the kidney, and increased echogenicity, which renders the ultrasound pattern brighter than the pattern of the liver (Courtesy of Dr. U. Vester, Essen). (**c**) Renal histology of nephronophthisis. Note the characteristic triad of tubular basement membrane disruption, tubular cell atrophy with cysts, and interstitial infiltration with fibrosis. Hematoxylin/eosin stain (Courtesy of Prof. R Waldherr, Heidelberg)

medullary cystic kidney disease (MCKD) [7, 10], due to similarities of clinical and pathologic features [24]. Both NPHP and MCKD feature corticomedullary cysts in the kidneys of normal or slightly reduced size. However, MCKD is clearly distinct from NPHP regarding multiple aspects: [1] MCKD follows autosomal dominant inheritance, [2] ESKD occurs in the fourth decade or later, and, [3] in MCKD, there is no extrarenal involvement other than hyperuricemia and gout (see ▶ Chap. 53, "Autosomal Dominant Tubulointerstitial Kidney Disease").

Nephronophthisis and related ciliopathies seem to be distributed evenly among males and females. Nephronophthisis has been reported from virtually all regions of the world [25] with an incidence of nine patients/8.3 million [26] in the United States or 1 in 50,000–80,000 live births in Canada [27] and Finland [28], respectively. The condition constitutes one of the most frequent genetic causes for ESKD in the first three decades of life and is a major cause of ESKD in children, accounting for ~ 5% of pediatric patients requiring renal replacement therapy in Europe [29]. In the North American pediatric ESKD population, pooled data indicate a prevalence of less than 5% [30]. MCKD appears to be somewhat less frequent.

NPHP was first described by Smith and Graham in 1945 [2] and by Fanconi et al. [3], who introduced the term "familial juvenile nephronophthisis." In NPHP, the earliest presenting symptoms are polyuria, polydipsia, decreased urinary concentrating ability, and secondary enuresis.

They occur in over 80% of cases [25] and start at around 6 years of age. In these early stages, regular fluid intake at nighttime is a characteristic feature of the patients' history. Anemia and growth retardation develop later in the course of the disease [31]. Due to the mild nature of symptoms and the lack of edema, hypertension, and urinary tract infections, there is often a delay in the diagnosis of NPHP. This may cause a risk of sudden death from fluid and electrolyte imbalances. Disease recurrence has never been reported in the kidneys transplanted to NPHP patients [32]. The early genes identified to cause NPHP, if mutated, were detected by positional cloning. Subsequently, advances in next-generation sequencing and bioinformatics have facilitated the identification of more than 90 different genes mutated in NPHP and related ciliopathies. This has made definite molecular genetic diagnostics possible (www.renalgenes.org). Mode of inheritance in NPHP-RC is strictly recessive, and as expected in a recessive disease, phenotypic penetrance seems to be 100%. However, there are allelic differences regarding disease severity and expected frequencies of distinct organ manifestations. Genes that may also cause non-syndromic NPHP have been numbered as *NPHP* genes *1–20* (www.omim.org, OMIM® Copyright® 1966–2020 Johns Hopkins University). Homozygous deletions in the *NPHP1* gene account for approximately 25% of NPHP cases [33], whereas mutations in any of the other NPHP-RC genes are each relatively rare.

In NPHP, chronic kidney disease develops before the age of 25 years [8, 34, 35]. Infantile NPHP, which is caused by mutations in *NPHP2/inversin*, leads to ESKD between birth and 3 years of age [6, 35]. In a study conducted in 46 children with juvenile NPHP type 1 caused by mutations of the *NPHP1* gene, a serum creatinine of 6 mg/dl was reached at a median age of 13 years of age (range 4–20 years) (Fig. 1a) [7, 13]. The median time lapse between a serum creatinine of 2 and 4 mg/dl was 32 months and between 4 and 6 mg/dl 10 months (Fig. 1a) [36]. Similarly, the median age of ESKD in patients with mutations in the *NPHP5* gene was 13 years [37]. In patients with adolescent NPHP due to mutations in the *NPHP3* gene, ESKD developed by 19 years of age [8]. If renal failure has not developed by the age of 25 years, the diagnosis of recessive NPHP should be questioned and autosomal dominant MCKD/tubulointerstitial kidney disease (ADTKD) should be considered as a differential diagnosis. In MCKD/ADTKD, ESKD occurs later in life. MCKD types 1 and 2 show a median onset of ESKD at 62 years [38] and 32 years [39], respectively.

Pathology and Histopathology

Renal histopathology is very similar in NPHP and classic MCKD and has been described comprehensively in 27 patients with NPHP by Waldherr and associates [10, 11] (Fig. 1c). Kidney size is normal or moderately reduced. There is always bilateral renal involvement. Macroscopically, the kidney surface has a finely granular appearance, most likely due to the protrusion of dilated cortical collecting ducts. There are between 5 and approximately 50 cysts of 1–15 mm in diameter, located preferentially at the corticomedullary border. The cysts arise primarily from the distal convoluted and medullary collecting tubules as shown by microdissection [40] but may also appear in the papilla. Cysts are observed only in about 70% of autopsy cases and seem to arise late in the course of the disease [41]. Therefore, the presence of cysts is not a prerequisite for diagnosis. No cysts are present in organs other than the kidney. The histologic changes of NPHP are characteristic but not specific for the disease and seem to develop only postnatally. Typically, there is pronounced thickening and multilayering of the tubular basement membrane (TBM), which represents the most characteristic histologic feature of NPHP (Fig. 1c). By light microscopy, there appears to be a specific sequence of events: TBM disruption is followed by lymphocytic and histiocytic peritubular infiltration. Subsequently, atrophic or dilated and tortuous tubules develop predominantly at the corticomedullary junction. In advanced stages, the picture merges into a diffuse sclerosing tubulointerstitial nephropathy. TBM changes and cyst formation are most prominent in distal tubules, where cysts are lined with a single layer of cuboidal or flattened epithelium [42–45]. Glomeruli demonstrate periglomerular fibrosis with splitting and thickening of Bowman's capsule. Glomerular obsolescence is only present in nephrons that have been destroyed by tubular alterations. On transmission electron microscopy, the tubular basement membrane shows thickening, splitting, attenuation, and granular disintegration without clear stages of transition [11]. A marked increase of microfilaments is seen at the base of the tubular epithelial cells.

Molecular Genetics and Disease Mechanisms

A positional cloning approach was used to gain insight into the pathogenesis of NPHP. This has revealed recessive mutations in nine different novel genes as causing NPHP. These are *NPHP1* [34, 46], *NPHP2/inversin* [6], *NPHP3* [47], *NPHP4* [48, 49], *NPHP5* [37], *NPHP6/CEP290* [50, 51], *NPHP7/GLIS2* [52], *NPHP8/RPGRIP1L* [53–55], and *NPHP9/NEK8* [56], defining NPHP types 1–9, respectively (Table 1).

Subsequently, genetic mapping, whole-exome sequencing (WES), and recently whole-genome sequencing have accelerated gene identification and led to the identification of 20 genes that may cause isolated NPHP, known to date. These define NPHP types 1–20. Additionally, more than 90 causative genes within the group of nephronophthisis-related ciliopathies (NPHP-RC) have been identified as causing syndromic ciliopathies that may include NPHP or related renal phenotypes (Table 1). All NPHP-RC genes are monogenic recessive (Mendelian) genes, implying that biallelic mutations in each single gene are in itself sufficient to cause NPHP by about 20 years of age, indicating that their gene products are necessary for normal kidney function. Gene identification has thereby generated new insights into normal function of tubular kidney epithelia and into disease mechanisms of NPHP and has revealed that molecular pathogenesis is related to signaling mechanisms of primary cilia, centrosomes, and planar cell polarity [1, 6, 57, 58] (see below and Fig. 2).

Identification of over 90 NPHP-RC-causing genes revealed multiple features as characteristic of NPHP-RC (Hildebrandt, 2011) (Table 1):

- NPHP-RC involves multiple organs (pleiotropy), depending on the mutated gene and allele.
- NPHP-RC proteins participate in protein complexes ("NPHPomes"), which dynamically localize to cilia, centrosomes, or the mitotic spindles in a cell cycle-dependent manner [20, 59].
- There is strong evolutionary conservation of NPHP proteins.
- Animal models (mice, zebrafish, *C. elegans*) are faithful disease models for NPHP-RC.
- NPHP-RC proteins participate in diverse signaling pathways (Wnt, Shh, hippo, mTOR, DNA damage response signaling, and cell cycle regulation) with the centrosome as an integrator.

Gene identification has made definite molecular genetic diagnostics possible (www.renalgenes.org) for approximately 50–70% of cases, depending on the composition of the cohort. Familial occurrence, parental consanguinity, early onset, and syndromic phenotypes are factors that suggest monogenic disease causation and increase the likelihood for positive results in genetic testing [60, 61]. Due to the large number of NPHP-RC genes and their overlapping clinical phenotypes, whole exome sequencing is the preferred technique for genetic diagnostics [62, 63]. However, gene panels by high-throughput multiplex PCR have also been used successfully [64]. Homozygous deletions in the *NPHP1* gene account for approximately 21% of all NPHP cases, thus representing by far the most common cause of NPHP. In comparison, mutations in all other genes are very rare, some occurring in less than ten families worldwide. The phenomenon of different gene mutations that all result in a similar phenotype is defined as genetic heterogeneity. Presently, the causative gene remains undetected in about 30% of cases, indicating that additional novel NPHP genes are yet to be identified. Some reports have suggested oligogenic inheritance in NPHP [65] and related ciliopathies [66–68], describing that more than one recessive gene may be mutated in some NPHP patients.

Table 1 Single-gene[a] causes of nephronophthisis-related ciliopathies (NPHP-RC). The table lists genes, protein complexes[b] ("NPHPomes"), OMIM[c] number for NPHP type, signaling mechanisms involved in the pathogenesis, organ involvement, syndromic spectrum (Joubert, Meckel, or Jeune syndromes), and animal models of NPHP-RC

				Organ involvement and syndromes [grey=present; black=prominent]							Animal models		
Genes mutated in NPHP-RC	Protein complex, "NPHP-omes" (NPHP protein numbers given)	NPHP type	Signaling pathway implicated in disease mechanism	Kidney	Retina	Liver	Neurologic/ Cerebellum	Bone	*Situs inversus*	Meckel syndrome	Mouse	*Zebra-fish, Xeno-pus*	*C. Ele-gans*
NPHP1	1-4-8	1	Wnt	▒	▒						*Nphp1*$^{-/-}$		*RNAi*
NPHP4	1-4-8	4		■	■								*RNAi*
RPGRIP1L	1- 4-8	8		▒	▒	▒	▒	■	■	■	*Ftm*		*kd*
INVS	2-3-9-AN	2	Wnt	▒	▒	▒					*Invs*$^{-/-}$, *Inv*	*kd*	*gk653*
NEK8	2-3-9-AN	9	DDR, Hippo	▒	▒						*Jck, Jcpk*	*kd*	
ANKS6	2-3-9-AN	16	HIF1α, Hippo, RNA binding	▒		▒			heart		*Cy rat, Anks6*$^{-/-}$	*kd, Xt*	
NPHP3	2-3-9-AN	3		▒	▒	▒	▒	■	■	■	*Nphp3*$^{-/-}$		
IQCB1	5-6-ATXN10	5		■	■							*kd*	
CEP290	5-6-ATXN10	6		▒	▒	▒	▒	■	■	■	*Rd16,(rdAc) Cep290*$^{-/-}$	*kd*	
ATXN10	5-6-ATXN10			▒		▒					*Sca10*		
SDCCAG8	10-OFD1	10	DDR	■	■		▒		PCD		*Sdccag8*$^{-/-}$	*kd*	
OFD1	10-OFD1		DDR				▒			SGB	*Ofd1*$^{-/-}$	*kd*	
MKS1	MKS1-6-						▒	■	■	■	*Kerouac*		*mks-1*
CC2D2A	MKS1-6-			▒	▒	▒	▒	■	■	■	*Cc2d2a*$^{-/-}$	*kd*	
TMEM231	MKS1-6-						▒	■	■	■	*Tmem231*$^{-/-}$		
TMEM67/MKS3		11		▒	▒	■	▒	■	■	■	*Wpk rat*		*Tm-2547*
TMEM216						▒	■					*kd*	
TCTN1	MKS1-6-						▒				*Tctn1*$^{-/-}$		
TCTN3							▒	■	■	■ OFD-IV			
TCTN2							■	■	■	■			
B9D1								■	■	■	*B9d1*$^{-/-}$		*b9d1*
B9D2								■	■	■	*B9d2*$^{-/-}$	*kd*	*b9d2*
IFT43	IFT-A		IFT, Shh										
IFT122	IFT-A		IFT, Shh										
IFT121/WDR35	IFT-A		IFT, Shh										
IFT144/WDR19	IFT-A	13	IFT, Shh	▒	▒								
IFT139/TTC21B	IFT-A	12	IFT, Shh				▒				*THM1, alien*$^{-/-}$		
IFT140	IFT-A		IFT, Shh	■	■	▒							
DYNC2H1	IFT-A							■			*Dync2h1*$^{-/-}$		
IFT52	IFT-B		IFT, Shh					■					
IFT80	IFT-B		IFT, Shh					■				*kd*	
IFT81	IFT-B		IFT, Shh	▒	▒			▒				*kd*	
IFT172	IFT-B	17	IFT, Shh	■	▒	▒	▒	■			*Wim, Avc*	*db,moe*	*osm-1*
CEP164		15	DDR, cell cycle regulation	▒	▒		▒				*Cep164*$^{-/-}$	*kd*	
INPP5E				▒	▒		■				*Inpp5e*$^{-/-}$	*kd*	
ARL13B							■				*Arl13b*$^{-/-}$	*sco*	*arl13b*
ZNF423		14	DDR	■	■		▒				*Zfp423*$^{/}$*, Nur12*		
AHI1				▒	▒		■				*Ahi1*$^{-/-}$	*kd*	
GLIS2		7	Shh								*Glis*$^{-/-}$		
KIF7							■				*Kif7maki*	kd	osm-3
TMEM138							■					*kd*	
TMEM237							■					*kd*	
CEP41							■				*Cep41*$^{-/-}$	*kd*	
CSPP1							■	▒		■			
CEP83		18		■			▒				*Cep83*$^{-/-}$		
DCDC2		19		▒		■					*Dcdc2*$^{-/-}$	*kd*	
MAPKBP1		20		▒									
21 BBS genes	BBSome			■	■	▒	■	■			*Bbbs1*^{M390R}, *ko*	*kd*	*RNAi*

BBS Bardet-Biedl syndrome, *DDR* DNA damage response signaling, *HIF1α* hypoxia-inducible factor-1, *hippo* hippo signaling, *IFT* intraflagellar transport, *kd* knockdown, *ko* knockout, *OFD* oral-facial-digital syndrome, *PCD* primary ciliary dyskinesia, *SGB* Simpson-Golabi-Behmel syndrome, *Shh* sonic hedgehog signaling, *SRP* short rib polydactyly syndrome, *Wnt* Wnt signaling

[a] All disease genes are recessive

[b] Sang et al. *Cell* 2011;145(4):513-28

[c] OMIM: http://www.ncbi.nlm.nih.gov/omim/?term=nphp*

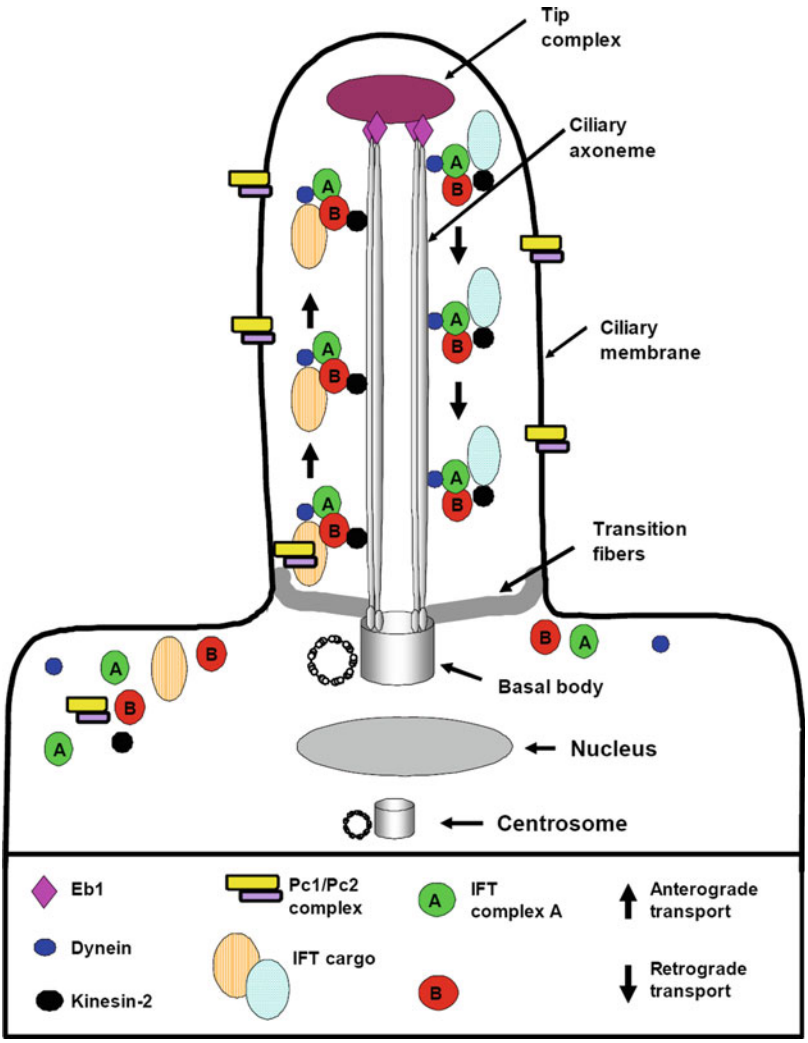
Tip complex
Ciliary axoneme
Ciliary membrane
Transition fibers
Basal body
Nucleus
Centrosome
A
B
Eb1
Pc1/Pc2 complex
IFT complex A
Anterograde transport
Dynein
IFT cargo
Retrograde transport
Kinesin-2

Fig. 2 Schematic structure of primary cilia and intraflagellar transport. The cilium is a hairlike structure that extends from the cell surface to the extracellular space. Virtually all vertebrate cell types can produce cilia. Cilia consist of a microtubule-based axoneme covered by a specialized plasma membrane. The axoneme has nine peripheral microtubule doublets. There may be two central microtubules (9 + 2 vs. 9 + 0 axoneme). 9 + 2 cilia usually have dynein arms that link the microtubule doublets and are motile, while most 9 + 0 cilia lack dynein arms and are nonmotile ("primary cilia"). The ciliary axoneme is anchored in the basal body, a microtubule-organizing center derived from the mother centriole. The transition zone at the junction of the basal body acts as a filter for any molecules that pass into or out of the cilium. Nephrocystin-1 is localized at the transition zone of epithelial cells [84]. During ciliogenesis, cilia elongate from the basal body by the addition of new axonemal subunits to the distal tip, the plus end of the microtubules. Axonemal and membrane components are transported in macromolecular particles (complexes A and B) by so-called intraflagellar transport (IFT) along the axonemal doublet microtubules [85]. Anterograde transport toward the tip is driven by heterotrimeric kinesin-2, which contains motor subunits Kif3a and Kif3b and a non-motor subunit. Mutations of *Kif3a* cause renal cysts and cerebellar vermis aplasia in mice [86]. Retrograde transport back to the cell body occurs via the motor protein cytoplasmic dynein 1b [87]. (Modified from Bisgrove and Yost, 2006 [88])

In the following, central aspects of the pathogenesis of NPHP will be discussed in the context of the discovery of NPHP genes 1–20 (Table 1). The structure and function of primary cilia and basal bodies are delineated in Fig. 2.

NPHP1

In juvenile nephronophthisis (NPHP type 1), a gene locus was mapped to chromosome 2q12.3 [69]. This locus was further refined [70–74], and the gene *NPHP1* responsible for NPHP type 1 was identified by positional cloning [34, 46]. About 85% of patients with NPHP type 1 carry large homozygous deletions of the *NPHP1* gene [75, 76]. Spontaneously occurring deletions of the *NPHP1* locus [77] as well as specific loss-of-function point mutations of *NPHP1* have been identified [34, 78]. In a subset of patients with large deletions in *NPHP1*, there is an association with oculomotor apraxia type Cogan [76]. Another subset shows an association with retinitis pigmentosa [78]. *NPHP1* encodes nephrocystin-1, a protein that interacts with components of cell-cell and cell-matrix signaling, including p130Cas [79], focal adhesion kinase 2 [79], tensin, and filamins A and B [80]. It is located at adherens junctions and focal adhesions of renal epithelial cells [81], which are involved in cell-cell and cell-basement membrane contacts, respectively (Fig. 3). Nephrocystin-1 also interacts with proteins encoded by other NPHP genes such as nephrocystin-2/inversin [6], nephrocystin-3 [47], and nephrocystin-4 [48, 49]. Nephrocystin-1 and nephrocystin-4 interact and colocalize with microtubules in primary cilia and at mitotic spindle poles, suggesting that nephrocystin may also play a role in cell division [82]. Knockdown of *NPHP1* resulted not only in defects of cilia formation but also in impaired assembly of tight junctions and epithelial cell polarity [83]. These data indicate that loss of cell orientation and alterations in cell division due to NPHP gene mutations may be two mechanisms that contribute to cyst formation in affected kidneys (Fig. 4).

NPHP2/Inversin

Infantile nephronophthisis (NPHP type 2) was recognized as a distinct disease entity, in which end-stage renal failure occurs within the first 3 years of live [5, 89] (Table 1). Macroscopically, NPHP type 2 differs from other forms of NPHP by the presence of enlarged kidneys and cortical microcysts and by the absence of medullary cysts. Histologically, there is no disruption of tubular basement membranes. Mutations of *NPHP2/INVS* were identified as the cause of infantile NPHP (type 2) with and without *situs inversus* [6] by positional cloning [35] and using candidate gene data [90, 91]. The renal cystic changes of infantile nephronophthisis combine clinical features of NPHP and of PKD [5]. The gene products nephrocystin-1 (NPHP1) and NPHP2/inversin interact with β-tubulin, which constitutes the microtubule axoneme of primary cilia (Fig. 2), and they are localized at primary cilia of renal tubular cells (Fig. 3) [6]. These findings supported a unifying theory of renal cystogenesis [1, 57, 92, 93], which states that proteins mutated in renal cystic disease (“cystoproteins”) in humans, mice, or zebrafish localize to primary cilia, basal bodies, or centrosomes in a cell cycle-dependent way [6, 57]. Basal bodies are the foundations from which cilia are assembled. Once mitosis and cell division are completed, basal bodies derive from the *mother* centriole that had previously organized the mitotic spindle in cell division. When cilia are formed from the basal body, the *daughter* centriole is placed on the side of the nucleus opposite to the basal body, thus specifying cell polarity (Fig. 3). Primary cilia are highly conserved structures that sense extracellular cues in a broad spectrum of epithelial tissues. There is a wide range of cues that can be received by specific ciliary receptors, including photosensation, mechanosensation, osmosensation, and olfactory sensation. In general, it seems that the pathogenesis of ciliopathies is based on an inability of epithelial cells to sense or process extracellular cues [94]. Similar to other cystoproteins, inversin was shown to localize to different subcellular locations in a cell cycle-dependent manner. Specifically, it is found at the mitotic spindle in mitosis; at the midbody in cytokinesis; and in cilia, their basal body, or centrosomes in interphase (Fig. 3). All of these

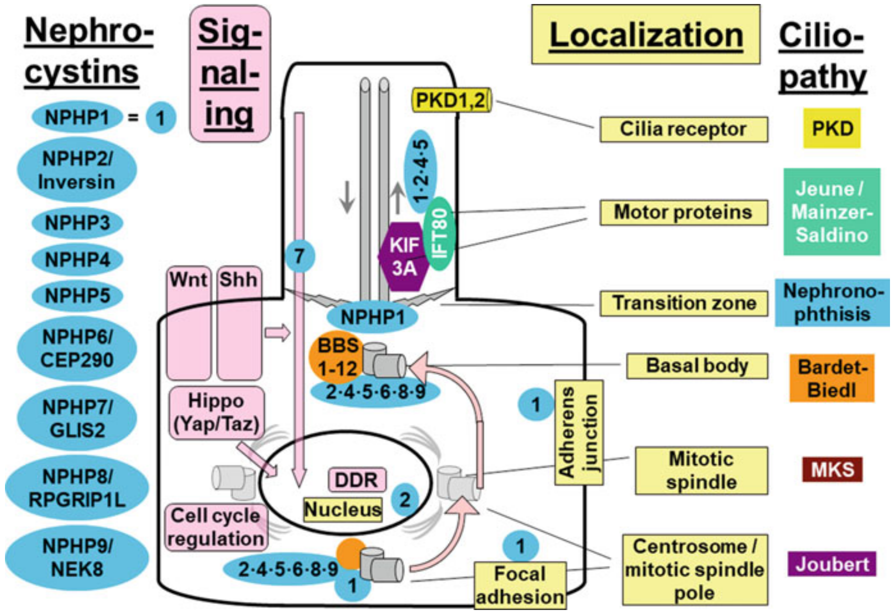
Nephro-cystins
NPHP1 = 1
NPHP2/Inversin
NPHP3
NPHP4
NPHP5
NPHP6/CEP290
NPHP7/GLIS2
NPHP8/RPGRIP1L
NPHP9/NEK8
Sig-nal-ing
PKD1,2
1·2·4·5
IFT80
KIF 3A
7
Wnt
Shh
NPHP1
BBS 1-12
2·4·5·6·8·9
Hippo (Yap/Taz)
1
Adherens junction
DDR
Nucleus
2
Cell cycle regulation
2·4·5·6·8·9
1
1
Focal adhesion
Localization
Cilia receptor
Motor proteins
Transition zone
Basal body
Mitotic spindle
Centrosome / mitotic spindle pole
Cilio-pathy
PKD
Jeune / Mainzer-Saldino
Nephrono-phthisis
Bardet-Biedl
MKS
Joubert

Fig. 3 Subcellular localization of nephrocystins to primary cilia, basal bodies, the mitotic spindle, focal adhesions, and adherens junctions, as well as functional interaction with other proteins mutated in renal "ciliopathies." "Cystoproteins" are proteins of genes mutated in cystic kidney diseases of humans, mice, or zebrafish. Depending on cell cycle stage, cystoproteins are localized at different subcellular organelles (shown in gray) [50, 96] including primary cilia, basal bodies, the endoplasmic reticulum, the mitotic spindle, centrosomes, adherens junctions, or focal adhesions. Arrows in the primary cilium indicate the direction of anterograde transport along the microtubule system mediated by kinesin-2 and retrograde transport by cytoplasmic dynein 1b. Most nephrocystins (blue) are located at cilia, the basal body, and centrosomes in a cell cycle-dependent manner. NPHP1 is also at the transition zone, focal adhesions, and adherens junctions. NPHP2/inversin and NPHP7/Glis2 can enter the nucleus and act as transcription factors. Sensory cilia perceive and process external signals, and "cystoproteins" are involved in signaling mechanisms downstream of ciliary signal recognition (pink). Downstream of cilia, Wnt signaling plays a role in planar cell polarity, which is mediated partially through orientation of centrosomes and the mitotic spindle poles. Cilia and ciliary proteins are functionally connected to the hedgehog and hippo signaling pathway. DNA damage response signaling (DDR), disrupted in some ciliopathies, may result in apoptosis and tissue degeneration. "Cystoproteins" with centrosomal/mitotic spindle localization may interfere with cell cycle regulation. Cilia-dependent mechanisms of planar cell polarity seem to be central to the pathogenesis of the ciliopathies, the most prominent of which is listed on the right. *Wnt* the Wnt signaling pathway, *Shh* the sonic hedgehog signaling pathway

organelles are involved in regulation of planar cell polarity or the cell cycle (see below). In this context, a major breakthrough was made for the understanding of the pathogenesis of renal cystic diseases when Simons et al. demonstrated a role of inversin/NPHP2 in signaling mechanisms of planar cell polarity, in particular Wnt signaling, necessary to maintain normal tubular development and morphology [95]. The proposed link between cell cycle defects, disruption of apical-basolateral polarity, and renal cyst formation is schematically summarized in Fig. 4 [58].

NPHP3

In a large Venezuelan kindred, we used positional cloning to identify missense mutations in *NPHP3* as responsible for adolescent nephronophthisis (Table 1) [8, 47]. Analogously, it was demonstrated that mutations in the mouse ortholog *Nphp3* are responsible for the renal cystic mouse mutant *pcy* [47], which was demonstrated to be responsive to treatment with a vasopressin receptor antagonist [97]. Missense mutations are mutations in which one single amino acid of the encoded protein is replaced by another amino

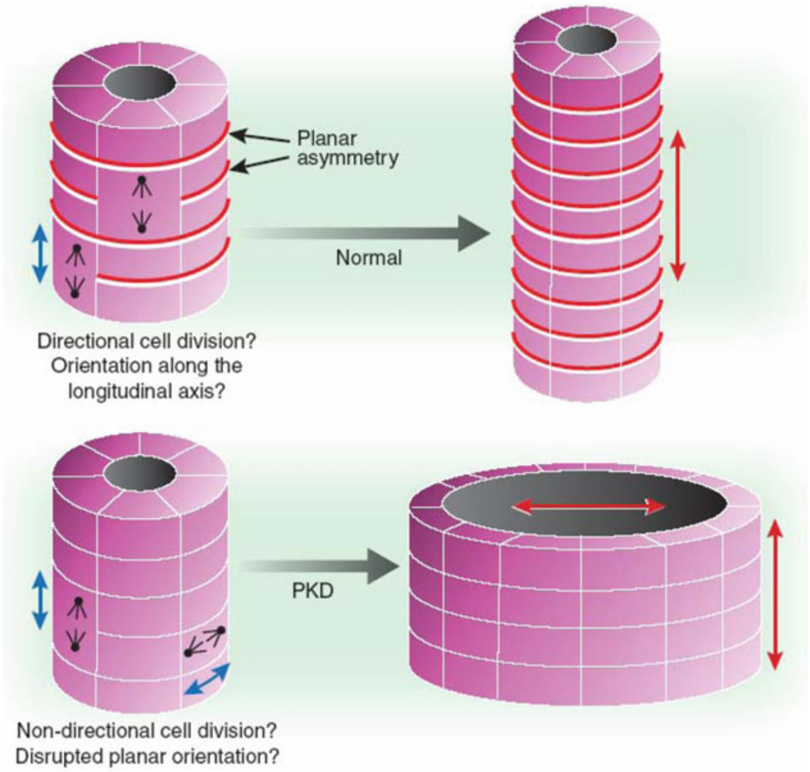
Planar
asymmetry
Normal
Directional cell division?
Orientation along the
longitudinal axis?
PKD
Non-directional cell division?
Disrupted planar orientation?

Fig. 4 Defects of cystoproteins lead to disruption of planar cell polarity and thereby to renal cysts through malorientation of the centrosome or the mitotic spindle. Correct orientation of the mitotic spindle and centrosomes with respect to the longitudinal axis of the tubule is critical for proper planar cell polarity (i.e., the orientation of an epithelial cell layer in three-dimensional space). Noncanonical Wnt signaling is involved in regulation of planar cell polarity during renal tubular morphogenesis, when in rodents 2 weeks *post-partum* the tubules still elongate. The structure that would result from disruption of this longitudinal orientation is a dilated tubule or cyst. (Adapted from [58])

acid. In contrast, truncating mutations introduce a premature stop codon and result in a shortened protein that is frequently degraded by mechanisms of nonsense-mediated decay. Therefore, truncating mutations are more likely to convey complete loss of function of the respective protein, while missense mutations may lead to partial residual protein function. In mice, complete loss of NPHP3 function results in *situs inversus*, congenital heart defects, and embryonic lethality [98]. Similarly, truncating mutations of *NPHP3* in humans can cause a clinical pattern that resembles Meckel syndrome. The phenotype of these patients included *situs inversus*, polydactyly, central nervous system malformations, structural heart defects, preauricular fistulas, and congenital anomalies of the kidney and urinary tract (CAKUT) [98]. A correlation between the “severity” of the underlying mutation and the resulting clinical phenotype has been observed for many NPHP genes. The notion that different mutations in the same gene can cause variable phenotypes is described as multiple allelism or phenotypic heterogeneity. Interestingly, truncating mutations of *NPHP3* give rise to developmental defects of the kidney, while patients with missense mutations have apparently normal kidneys at birth and only later in life develop degenerative renal disease. Thus, for NPHP-RC genes, there appears to be a phenotypic continuum from developmental defects to degenerative diseases of various organs.

NPHP4

Mutations in the gene *NPHP4* were identified by homozygosity mapping and total genome search for linkage [48, 49, 99] (Table 1). Nephrocystin-4, like inversin, localizes to primary cilia, basal bodies, centrosomes, and the cortical actin cytoskeleton [82] (Fig. 3).

NPHP5

Recessive mutations in the gene *NPHP5* were identified as mutated in nephronophthisis type 5 [37]. All mutations detected resulted in truncations of the encoded protein nephrocystin-5, and all patients had an association with early-onset retinal degeneration. Thus, *NPHP5* represents the gene mutated in the classical early-onset form of Senior-Loken syndrome (SLS) (Table 1). Nephrocystin-5 contains an IQ domain, which directly interacts with calmodulin [37], and is in a complex with the retinitis pigmentosa GTPase regulator (RPGR), which when defective causes X-linked retinitis pigmentosa. Both nephrocystin-5 and RPGR are localized in connecting cilia of photoreceptors and in primary cilia of renal epithelial cells [37]. The fact that connecting cilia of photoreceptors are the structural equivalents of primary cilia of renal epithelial cells rendered an explanation for retinal involvement in the retinal-renal syndrome Senior-Loken syndrome.

NPHP6/CEP290

Recessive truncating mutations in the gene *NPHP6/CEP290* were identified as the cause of NPHP type 6 and Joubert syndrome type 5 by positional cloning [50]. Its gene product nephrocystin-6/CEP290 is part of the centrosomal proteome [100] (Table 1). Similar to NPHP2/inversin and NPHP4, NPHP6/CEP290 is expressed in centrosomes and the mitotic spindle in a cell cycle-dependent manner. Abrogation of *nphp6* function in zebrafish caused planar cell polarity defects and recapitulated the human phenotype of NPHP type 6, including renal cysts, retinal degeneration, and cerebellar defects [50]. Nephrocystin-6 modulates the activity of ATF4/CREB2, a transcription factor that may be implicated in cAMP-dependent renal cyst formation [101]. Interestingly, a 300-amino acid in-frame deletion of *Nphp6/Cep290* caused retinal degeneration only, without renal or cerebellar involvement in the *rds16* mouse model [102] (Table 1). This is in accordance with the recent finding that hypomorphic mutations of *NPHP6/CEP290* represent the most frequent cause of Leber's congenital amaurosis [103]. Mutations in *NPHP6/CEP290* have been confirmed as causing Joubert syndrome (JBTS, see below) with and without renal involvement [51]. Truncating mutations in *NPHP6* were shown to cause Meckel syndrome [104]. This is another example of multiple allelism in NPHP-RC.

Correct orientation of the mitotic spindle and centrosomes with respect to the longitudinal axis of the renal tubule is critical for proper apical-basolateral polarity (Fig. 4). Noncanonical Wnt signaling is involved in these processes in renal tubular morphogenesis, when in rodents renal tubules still elongate postnatally. The structure resulting from disruption of the longitudinal growth would be a dilated tubule or cyst (Fig. 4). Additional evidence for a role of planar cell polarity in renal cystic diseases was generated by measuring orientation of the mitotic spindle through 3-D imaging of renal tubules [105]. Comparison of the distribution of the mitotic angles in wild-type animals and rodent cystic kidney disease models revealed that mitotic angles of two rodent models of cystic kidneys, the HNF1β-deficient mouse model and the *pck* rat model, differed from wild-type littermates [105].

NPHP7/GLIS2

Mutations in the *NPHP7/GLIS2* gene, encoding the transcription factor Gli-similar protein 2 (Fig. 3), were discovered as the cause of NPHP type 7 [52]. In analogy, *Glis2* mutant mice showed severe renal atrophy and fibrosis resembling human nephronophthisis [52] (Table 1). Differential gene expression studies on *Glis2* mutant kidneys demonstrated that genes promoting epithelial-to-mesenchymal transition and fibrosis are upregulated in the absence of *Glis2* [52]. There was also prominent apoptosis present in distal tubular segments of the kidney, which might provide an explanation why, in contrast to PKD, kidney size is reduced in NPHP. As GLIS2 is related to the GLI transcription factor, these findings implicated the hedgehog signaling pathway in the pathogenesis of cystic kidney diseases [106]. Hedgehog is a signaling pathway that controls cell determination and tissue patterning during embryogenesis. Subsequently identified NPHP genes, in particular those associated with skeletal phenotypes (see below), further supported a role of hedgehog signaling in the pathogenesis of NPHP-RC.

NPHP8/RPGRIP1L

Missense and truncating mutations in the *NPHP8/RPGRIP1L* gene were shown to cause Joubert syndrome and Meckel syndrome, respectively [54] (Table 1). RPGRIP1L colocalized with the protein products of *NPHP6* and *NPHP4* at the basal body and centrosomes [54] (Fig. 3). While two truncating mutations caused Meckel syndrome, missense mutations were seen in patients with Joubert syndrome [54, 55]. These findings confirmed that there is a continuum for the phenotypic abnormalities found in Meckel syndrome, in Joubert syndrome, and in nephronophthisis on the basis of distinct mutations in the identical genes (multiple allelism).

NPHP9/NEK8

Three different highly conserved amino acid changes (missense mutations) were identified in the gene *NEK8* (never in mitosis kinase 8) as causing NPHP type 9 [56] (Table 1). One of the mutations identified in human patients affected the same RCC1 domain, in which the missense mutation causing the renal cystic mouse model *jck* is also positioned [107, 108]. The notion that mutations in *NEK8* cause nephronophthisis was further supported by cell culture experiments: Upon expression in medullary collecting duct cells, all three mutant forms of *NEK8* showed defects in subcellular localization; specifically, ciliary and centrosomal localization of mutant NEK8 was disrupted [56]. As NEK8 plays a major role in cell cycle regulation, these data provided further evidence for a functional link between renal cyst formation and cell cycle dysregulation. At the same time, it further supported the dual role of ciliary/centrosomal proteins as cell cycle regulators. In this context, it is interesting that two mouse models of polycystic kidney disease (*jck* and *cpk*) can be efficiently treated with the cyclin-dependent kinase inhibitor roscovitine [109].

ZNF423 and CEP164

Mutations in zinc finger protein 423 (*ZNF423*) and centrosomal protein 164 (*CEP164*) were identified as causing NPHP types 14 and 15, respectively [110]. These findings implicated the ataxia telangiectasia mutated (ATM) branch of DNA damage response (DDR) signaling in the pathogenesis of NPHP-RC [111]. A role of DDR was then confirmed for the fanconi anemia 1 (FAN1) branch of DDR in NPHP-RC like disease “karyomegalic interstitial nephritis” [112] and for the ataxia telangiectasia and Rad3-related (ATR) branch of DDR by the description of DDR defects in a mouse model of *Nek8/Nphp9* loss of function [113]. Genomic integrity in mammalian cells is closely monitored by the DDR signaling network, which, upon detection of DNA damage, induces cell cycle arrest to facilitate DNA repair. In conditions of profound DNA damage, when repair fails, apoptosis is initiated to eliminate defective cells and maintain genomic integrity. Experimental studies in cell culture, zebrafish, and mice demonstrated that in addition to their canonical function in cilia and centrosomes, some NPHP proteins carry additional functions in DDR regulation [110, 114, 115]. Dysregulation of DDR signaling in patients with NPHP-causing mutations may contribute to the development of renal fibrosis by inducing apoptosis and epithelial-to-mesenchymal transition with concomitant fibrosis in renal epithelial cells [110, 114, 115] (Fig. 5). Centrosomal proteins, such as CEP164 and CEP290, thus have multiple cellular functions, i.e., in cilia, cell cycle regulation, and DDR signaling that may collectively contribute to NPHP pathogenesis (Fig. 3).

WDR19/NPHP13, IFT172/NPHP17, and Other Genes Encoding Intraflagellar Transport (IFT) Proteins

Mutations in *WDR19*, encoding the intraflagellar transport protein 144 KDa (IFT144), were found

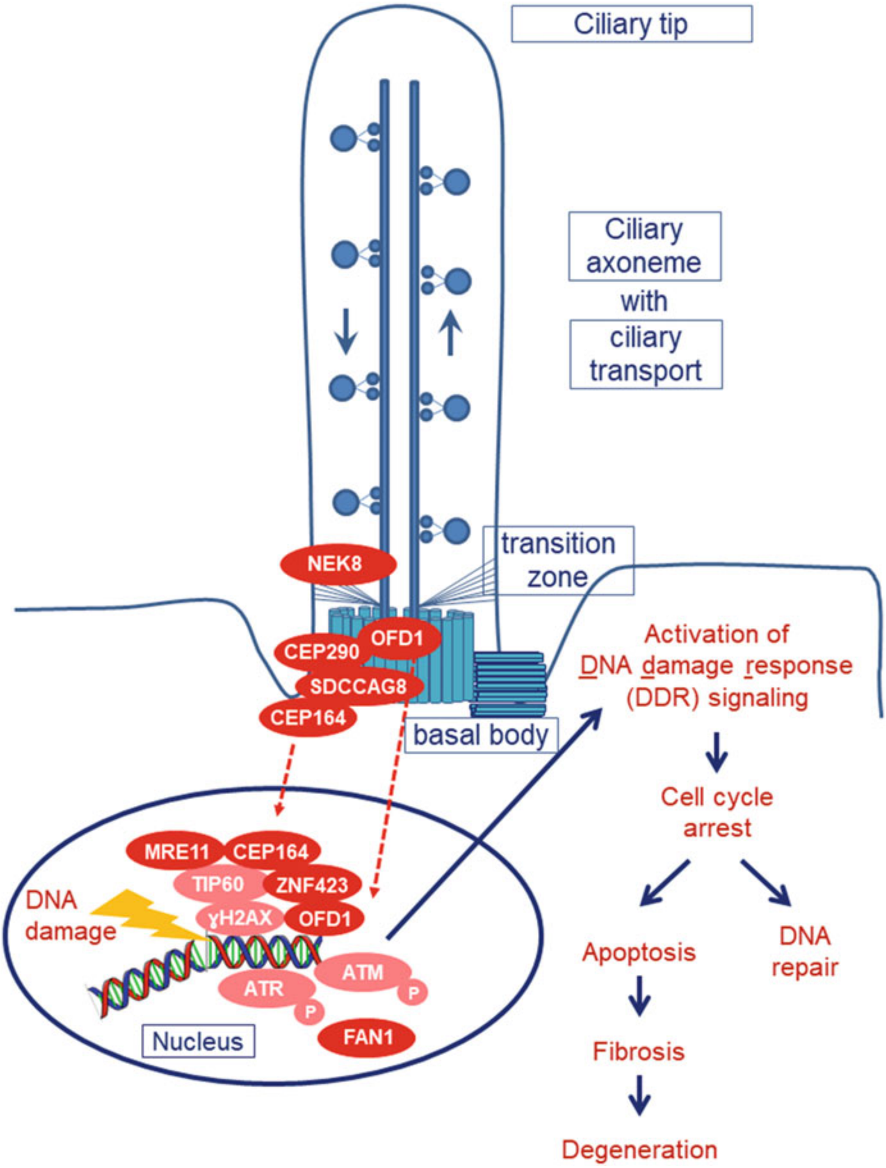
Ciliary tip
Ciliary axoneme
with
ciliary transport
transition zone
NEK8
OFD1
CEP290
SDCCAG8
CEP164
basal body
Activation of DNA damage response (DDR) signaling
Cell cycle arrest
Apoptosis
DNA repair
Fibrosis
Degeneration
MRE11
CEP164
TIP60
ZNF423
γH2AX
OFD1
DNA damage
ATM
ATR
P
P
FAN1
Nucleus

Fig. 5 Primary cilia play a critical role in numerous signaling pathways, among them DNA damage response (DDR) signaling. In events of DNA damage, several centrosomal proteins translocate to nuclear foci where they colocalize with the TIP60 complex. A number of ciliopathy proteins harbor this dual function and modulate different branches of the DDR signaling network. In ciliopathies, dysregulation of DDR signaling in renal epithelial cells may induce apoptosis and concomitant tissue fibrosis, thereby driving the degenerative renal phenotype of NPHP, which significantly differs from PKD with respect to pathogenesis and histology. Light red defines essential proteins of the DDR pathway; dark red defines NPHP proteins that are functionally connected to DDR signaling. Abbreviations: ATR, ataxia telangiectasia and Rad3 related; ATM, ataxia telangiectasia mutated; FAN1, fanconi anemia 1; ATR/ATM/FAN1, different branches of DDR signaling; γH2Ax, gamma histone-H2AX

in patients with cranioectodermal dysplasia, retinitis pigmentosa, and renal cysts [116, 117]. Subsequently, barcoded multiplex PCR-based screening in a large international cohort reported *WDR19* mutations in patients with NPHP with/without retinal degeneration [64]. Mutations in

IFT172 were identified as causing NPHP type 17 combined with short-rib polydactyly [22]. IFT proteins form two distinct multiprotein complexes, ITFA and IFTB, required for anterograde and retrograde transport along the ciliary axoneme, respectively (Fig. 2). Both IFT complexes are highly conserved across evolution from green algae to humans and function as adaptors between ciliary motor proteins, i.e., kinesins and dyneins, and their respective cargo. As cilia are sensory organelles required for sensing and processing of extracellular cues, ciliary transport of messenger molecules directed for the nucleus is essential for rapid signal transduction. Impairment of ciliary transport proteins due to mutations will presumably cause a block in a cell's ability to respond to external cues, thereby resulting in tissue dysplasia or degeneration, depending on the severity of the defect. Additionally, cilia do not contain any intrinsic machinery for protein biosynthesis, which might explain why genetic inactivation of IFT proteins in mice disrupts ciliogenesis (formation of cilia) [86]. Mouse models with lack of cilia due to IFT disruption developed renal cysts and thereby supported the proposed link between ciliary defects and renal cyst formation. Interestingly, patients with mutations in genes encoding IFT proteins mostly displayed skeletal ciliopathies, such as Jeune's asphyxiating thoracic dystrophy (JATD), short-rib polydactyly syndrome, Mainzer-Saldino syndrome, or Sensenbrenner syndrome (cranioectodermal dysplasia) (see below). This phenomenon is thought to result from disruption in the hedgehog signaling pathway [22, 118] (Fig. 3), a pathway that is essential for skeletal development. Additional organ manifestations in these patients varied depending on the mutated gene and the specific allele and included NPHP, retinal degeneration, liver fibrosis, and cerebellar vermis hypoplasia [22, 64, 117, 118].

ANKS6/NPHP16

Mutations in *ANKS6* were found in patients with NPHP type 16 and mostly infantile onset of renal cystic disease combined with congenital heart defects and congenital liver fibrosis [119], suggesting that ANKS6 was involved in renal, cardiac, and hepatic morphogenesis. ANKS6 was shown to localize to the proximal compartment of the ciliary axoneme, where it forms a multiprotein complex with three other "NPHP proteins," namely, NPHP3, INVS, and NEK8 [119] (Table 1). A knockout mouse model of *Anks6* displayed abnormal cilia as well defects in bile duct formation and cholangiocyte differentiation, which the authors found were related to disruptions in the hippo signaling pathway [120]. This study thereby defined another signaling network essential to organ development, which is functionally connected to cilia. A recent study furthermore detected RNA-binding properties in the ankyrin repeat domain of ANKS6 as well as inversin (NPHP2) and demonstrated that both proteins can translocate into RNA-containing granules in response to cellular stress. These findings support the observation that certain NPHP proteins act as intracellular sensors for conditions of genomic stress [121].

CEP83/NPHP18

Patients with homozygous or compound heterozygous mutations in *CEP83* had early onset of NPHP with rapid progression to ESKD. Additionally, some patients had hydrocephalus and/or variable degrees of intellectual disability [122]. *CEP83*, like *CEP164*, encodes a centrosomal protein that is an essential component of the distal appendage of centrioles (Fig. 2) and is required for docking of cargo-bearing vesicles from the Golgi apparatus to the cilium. Depletion of CEP83 thus impairs ciliogenesis by interfering with the first step of ciliary transport [123]. Mice with conditional knockout of *Cep83* in radial glial progenitor cells provided evidence for a role of the centrosome and centrosomal proteins in progenitor cell maintenance and patterning [124].

DCDC2/NPHP19

Mutations in *DCDC2* were first detected in patients with NPHP type 19 and early onset of liver fibrosis [20]. Subsequently, an isolated hepatic phenotype, namely, neonatal sclerosing cholangitis, was found to be caused by recessive mutations of *DCDC2* [125, 126] suggesting that DCDC2 carries an essential function in cholangiocytes and gives rise to a predominantly hepatic ciliopathy, which may include other organ manifestations. *Dcdc*2 knockout mice recapitulated the hepatic phenotype of affected patients and developed periportal liver fibrosis with abnormalities in biliary duct structure [20]. DCDC2 is a microtubule-binding protein that colocalizes with acetylated tubulin at the ciliary axoneme and mitotic spindle fibers in a cell cycle-dependent manner [20].

MAPKBP1/NPHP20

Mutations in *MAPKBP1* were shown to cause NPHP type 20, and extrarenal manifestations were not present in any of the identified patients [127, 128].

Animal Models

Several spontaneously occurring mouse models of mutated genes that exhibit an NPHP-like phenotype or a phenotype of SLS-like retinal degeneration were shown to represent orthologs of human NPHP genes. Examples are shown in Table 1, e.g., for the genes *NPHP2/INVS*, *NPHP3*, *NPHP6/CEP290*, *NPHP7/GLIS2*, *NPHP8/RPGRIP1L*, and *NPHP9/NEK8*. Furthermore, mouse models with generalized, or tissue-specific knockout of NPHP genes has been generated that reproduce renal, hepatic, neurological, skeletal, and/or developmental phenotypes of human ciliopathies. Zebrafish also recapitulate a wide array of human ciliopathy phenotypes and are therefore frequently applied as an animal model for nephronophthisis. Ciliopathy phenotypes in zebrafish include ventral body axis curvature and hydrocephalus, indicative of laterality defects as seen in humans with *situs inversus*. Zebrafish with knockdown of the orthologous genes of *CEP290/NPHP6* and *CEP164/NPHP15* displayed developmental brain defects, retinal dysplasia, and kidney cysts [110, 129]. As seen in patients with mutations of *IFT172*, zebrafish *ift172* morphants had skeletal defects [22]. Nephrocystin-4 is conserved in *C. elegans* and expressed in ciliated head and tail neurons of the nematode [130]. Upon knockdown, it exhibits a male mating phenotype, similar to the phenotype found upon knockdown of the polycystin-1 and polycystin-2 orthologs [131]. Localization of nphp-1 and nphp-4 to some of these ciliated neurons also overlaps with localization of the cystoprotein orthologs polycystin-1 (*lov-1*) and polycystin-2 (*pkd-2*) and with many orthologs of Bardet-Biedl syndrome (BBS) proteins [130, 132] similar to what has been described for *lov-1* and *pkd-2* mutants [131]. These data have been recently refined for specific neuronal cell types [130, 132], and the necessity of *nphp-1* and *nphp-4* for morphologic integrity of ciliated neurons in *C. elegans* was demonstrated [133, 134]. In addition, a role for *nphp-4* in life span of the worm has been demonstrated [135]. Evolutionary conservation of NPHP-RC proteins goes even further: Some have been conserved over more than 1.5 billion years of evolution from the unicellular organism *Chlamydomonas reinhardtii* to vertebrates. *Chlamydomonas reinhardtii* uses two motor cilia (flagella) for locomotion. Strikingly, nephrocystin-4 and at least six proteins mutated in BBS are conserved in *Ch. reinhardtii* where they constitute part of its basal body proteome [132, 136]. Defects of cystoprotein orthologs in *Ch. reinhardtii* have deficient intraflagellar transport and flagellar propulsion [137]. This further supports the notion that cystoproteins play a role in functional modules that are conserved throughout evolution.

Extrarenal Manifestations of NPHP

A prominent feature of NPHP is involvement of multiple organs in addition to the kidney (pleiotropy) (Table 1). Interestingly, organ manifestations of ciliopathies can be of developmental and degenerative nature. Infantile NPHP type 2 can be associated with *situs inversus* [6], liver fibrosis [138], or cardiac ventricular septal defect [6]. Furthermore, NPHP is frequently accompanied by tapetoretinal degeneration (Senior-Loken syndrome) [15, 16], cerebellar vermis hypo-/aplasia (Joubert syndrome) [17, 18], ocular motor apraxia type Cogan [139], mental retardation [50], liver fibrosis [19], or skeletal anomalies, such as cone-shaped epiphyses of the phalanges (Mainzer-Saldino syndrome) [21] or asphyxiating thoracic dystrophy (Jeune syndrome) [22, 140]. In some instances, there appears to be a genotype-phenotype correlation regarding pleiotropy and organ involvement. For example, there is involvement of the retina in most cases with mutations of *NPHP5* or *NPHP6*. Patients with mutations affecting IFT proteins mostly show prominent skeletal phenotypes. Patients with mutations in *ANKS6* and *DCDC2* are prone to developing liver fibrosis [20, 119].

Retinal Involvement (Senior-Loken Syndrome)

The renal-retinal involvement in Senior-Loken syndrome (SLS) can be explained by the fact that the primary cilium of renal epithelial cells is a structural equivalent of the connecting cilium of photoreceptor cells in the retina [141]. It was shown that nephrocystin-5 and nephrocystin-6 are expressed in the connecting cilia of photoreceptors [37, 102].

Cerebellar Vermis Hypoplasia (Joubert Syndrome)

In Joubert syndrome (JBTS), NPHP is associated with coloboma of the eye, with aplasia/hypoplasia of the cerebellar vermis causing ataxia, and with the variable symptoms of psychomotor retardation and episodic neonatal tachy-/dyspnea [17, 18, 142–144]. The radiographic feature of JBTS on axial magnetic resonance brain imaging is the so-called molar tooth sign of the midbrain-hindbrain junction [144, 145]. Ocular motor apraxia type Cogan, defined as the transient inability of horizontal eye movements in the first few years of life, may also be associated with JBTS. This symptom has been first described in patients with mutations in the *NPHP1* gene [76, 139] (causing JBTS type 4). To date, the monogenic causes of JBTS types 1–36 have been identified. Depending on the underlying mutated gene, involvement of other organ systems may be present (syndromic ciliopathies). There is multiple allelism for many JBTS genes, indicating that depending on the distinct mutation (allele), different ciliopathy phenotypes can arise, e.g., *CEP290* (JBTS type 5, NPHP type 6, SLS type 6, MKS type 4), *TMEM67* (JBTS type 6, NPHP type 11, MKS type 3, COACH syndrome), and *NPHP*1 (NPHP type 1, JBTS type 4, SLS type 1). Several JBTS genes are allelic to Meckel syndrome, a severe, embryonically lethal developmental ciliopathy characterized by cystic dysplasia of virtually all organ systems.

Liver Fibrosis

NPHP can be associated with periductal liver fibrosis [19, 146–148], as has been described for a patient with *NPHP3* mutation [47]. Furthermore, liver involvement is prominent in patients with mutations of *TMEM67* [149] (NPHP type 11), *ANKS6* [119] (NPHP type 16), and *DCDC2* [20] (NPHP type 19), which has also been described in patients with neonatal sclerosing cholangitis [125, 126]. The phenotypic spectrum of hepatic ciliopathies involves hepatomegaly, periportal fibrosis, and congenital hepatic fibrosis, as well as biliary dysgenesis and bile duct proliferation. Liver involvement is also present in autosomal recessive polycystic kidney disease (ARPKD). Bile duct involvement in these cystic kidney diseases is likely also explained by cilia-related mechanisms as the epithelial cells lining bile ducts (cholangiocytes) possess primary cilia [150].

Multi-organ Cystic Dysplasia with Severe Brain Malformations (Meckel Syndrome)

Within the spectrum of NPHP-associated ciliopathies, Meckel syndrome (MKS) is the most severe form. It leads to perinatal mortality with renal cystic dysplasia, occipital encephalocele, polydactyly, and biliary digenesis. Multiple recessive gene mutations have been identified, including in *MKS1* [151] and *MKS3* [152]. A Meckel-like phenotype has been described for truncating mutations of *NPHP3* [98], *NPHP6/CEP290* [104], and *NPHP8/RPGRIP1L* [54, 55]. MKS represents a ciliopathy that encompasses defects in most organs, and organ involvement is of developmental rather than degenerative nature. For instance, organ defects reveal cystic dysplasia of the kidneys, microphthalmia, liver bile duct dysgenesis, occipital encephalocele in the brain, and skeletal malformations, such as postaxial polydactyly are frequently seen [153]. The notion that MKS is at the most severe end of the clinical spectrum is supported by the finding that having two truncating mutations in *NPHP8/RPGRIP1L* causes MKS, whereas one "mild" mutation (missense rather than truncating) may cause the less severe phenotype of JBTS [104].

Cardiac Defects and *Situs Inversus*

In a patient with a mutation of *NPHP2*, a cardiac ventricular septal defect has been described [6]. Thus, the role of inversin for left-right axis specification known from mouse models was confirmed in humans [91, 154]. As this phenotype was associated with *situs inversus,* a cardiac ventricular septal defect may be viewed as a "heterotaxy" (left-right orientation) phenotype caused by the same mechanism [155]. The phenotypic combination of cystic kidney disease, *situs inversus*, and cardiac septal defects on the basis of *INVS* mutations has been observed in humans, mice, and zebrafish [6]. Congenital heart defects have also been observed in patients with *ANKS6* mutations [119]. Performing a forward genetic screen in mice, the group of Cecilia Lo identified mutations affecting ciliary proteins and/or ciliary signaling as a major pathogenic cause of congenital heart defects in mice [156], thereby strengthening the link between cilia and cardiac morphogenesis.

Skeletal Defects

NPHP can be associated with syndromic skeletal defects, including Jeune syndrome (asphyxiating thoracic dysplasia) [140, 157–159], Ellis-van Creveld syndrome [160], RHYNS syndrome (retinitis pigmentosa, hypopituitarism, NPHP, skeletal dysplasia) [161], Sensenbrenner syndrome (cranioectodermal dysplasia) [162, 163], and Meckel-Gruber syndrome [151, 152]. This strongly suggests a role of primary cilia in skeletal development. The association of NPHP with cone-shaped epiphyses of the phalanges is known as *Mainzer-Saldino syndrome* and was originally described in patients who also had retinal degeneration and cerebellar ataxia [21]. Interestingly, mutations in the ortholog of the intraflagellar transport protein IFT80 of *Chlamydomonas reinhardtii* were found to be the cause of *Jeune* syndrome [164] (Fig. 4), which emphasized the strong evolutionary conservation of "ciliopathy genes." When mutations in genes that encode other IFT proteins were identified in individuals with *Jeune*, *Mainzer-Saldino*, and *Sensenbrenner* syndromes, a link between IFT defects and skeletal ciliopathies was confirmed, most likely pointing toward an involvement of the sonic hedgehog signaling pathway in the pathogenesis of these ciliopathy phenotypes [22, 164, 165] (Table 1).

Bardet-Biedl Syndrome

Bardet-Biedl syndrome (BBS) is a syndromic ciliopathy characterized by pleiotropic organ manifestations and variable phenotypes that were first described by Laurence and Moon in 1866. To facilitate clinical diagnosis of BBS, diagnostic criteria have been established. Major criteria include renal anomalies, retinal degeneration (rod-cone dystrophy), obesity, polydactyly, intellectual disability, and hypogonadism. Minor criteria include cranioectodermal dysplasia (i.e., dental crowding, high-arched palate), cataracts, diabetes mellitus, hepatic fibrosis, congenital heart disease, and various neurological disorders (e.g., developmental delay, ataxia). The presence of four major criteria or the combination of three major and additional minor criteria defines the diagnosis of BBS. Studies have found renal involvement in >50% of BBS patients [166–168]. One British study in 350 BBS patients reported structural anomalies of the kidney in ~50% of patients and impaired renal function (CKD stages 2–5) in ~30% of pediatric patients and ~40% of adult patients with BBS [167]. The investigators observed that about 8% of BBS patients progressed to ESKD, mostly within the first five years of life [167]. Adult onset of CKD or ESKD was mainly from secondary causes, most notably type II diabetes mellitus and/or hypertension, which are frequent comorbidities in BBS patients. Structural anomalies of the kidney in BBS patients are diverse, including developmental malformations, hydronephrosis, interstitial fibrosis, and cystic dysplastic kidney disease. As observed in other ciliopathies, BBS is also characterized by genetic heterogeneity, and to date, mutations in 21 genes have been identified to cause BBS types 1–21. Some of those genes are allelic to other NPHP-RC (e.g., the genes *CEP290*, *TMEM67*, and *SDCCAG8*), while others have only been found in BBS patients. Mode of inheritance in

BBS is typically autosomal recessive; however, complex patterns, such as oligogenic inheritance, have been described [67]. Proteins encoded by BBS genes (i.e., BBS 1, 2, 4, 5, 7, 8, and 9) form a multiprotein complex (the BBSome, Fig. 3), which is localized at the ciliary base and is required for vesicle docking and ciliary entry of Golgi-derived proteins [169].

Diagnosis

Laboratory Studies

Patients with NPHP are often diagnosed only when an increased serum creatinine value is detected fortuitously. Patient history generally reveals prolonged nocturia since school age. Specific gravity of a morning urine specimen will be low. Renal ultrasound will then corroborate the diagnosis (Fig. 1b), which can be subsequently confirmed by molecular genetic diagnostics (www.renalgenes.org). A diagnostic algorithm for nephronophthisis has been suggested [170], but a positive diagnosis can now more easily be established by high-throughput exon sequencing [64] or whole-exome sequencing [62] (www.renalgenes.org). Hematuria, proteinuria, and bacteriuria almost never occur in NPHP, so that a bland urine sediment is a further diagnostic hint. Rarely, however, there can be an “overlapping” clinical phenotype presenting as a combination of NPHP-RC and nephrotic syndrome. In these instances, mutations have been detected in *ARHGDIA* [171] or *FAT1* [172].

Laboratory studies are needed to assess the severity of renal failure. Typically, patients have elevation of serum creatinine, blood urea nitrogen, and phosphorus, together with metabolic acidosis, hypocalcemia, electrolyte imbalance, and anemia. In SLS, retinitis pigmentosa is diagnosed by its specific findings on ophthalmoscopy including increased retinal pigment, attenuation of retinal vessels, and pallor of the optic disc. If retinitis pigmentosa is present, electroretinography and electrooculography can be used to evaluate severity. Retinal degeneration is characterized by a constant and complete extinction of the electroretinogram, preceding visual and fundoscopic signs of retinitis pigmentosa. Ophthalmoscopy should be performed in any patient with nephronophthisis to evaluate for signs of retinal degeneration. Laboratory assessment of liver function and hepatic ultrasonography are important to facilitate detection of patients with hepatic fibrosis. Skeletal involvement can be detected by thorough clinical examination. In patients with infantile NPHP, echocardiography is advisable.

Imaging

The most useful imaging technique in NPHP is renal ultrasonography. The kidneys are of normal or moderately reduced size and show increased echogenicity; loss of corticomedullary differentiation; and, in later stages, cyst formation at the corticomedullary border of the kidneys [9] (Fig. 1b). Garel and associates have described medullary cysts in 13 of 15 children studied at the time of renal failure (mean age 9.7 years) [173]. Roentgenography contributes little to the diagnosis of the disease. Medullary cysts can sometimes also be demonstrated on magnetic resonance imaging or computed tomography [174, 175]. Histology is characteristic but not pathognomonic in NPHP, since cysts may be absent and tubulointerstitial disease can be nonspecific. Renal biopsy can be circumvented as an initial procedure due to the availability of molecular genetic diagnostics in NPHP (www.renalgenes.org). If genetic diagnostics do not detect a molecular defect, the diagnosis can be based on the combined results of typical clinical history with polyuria, polydipsia, and anemia; the classical appearance of the kidneys on ultrasound; and renal histology. A thorough pedigree analysis should be documented for each of three successive generations, to rule out autosomal dominant MCKD/ADTKD.

Molecular Genetic Diagnostics

Knowledge of causative gene mutations, technical advances in next-generation sequencing technology, and cost reduction for genetic testing have made definite molecular genetic diagnostics possible (www.renalgenes.org) and applicable for routine diagnostics in clinical settings. In patients with the suspected diagnosis of NPHP-RC based on clinical presentation and/or renal ultrasound imaging, identification of the underlying gene mutation succeeded in up to 50–70% of cases through application of high-throughput barcoded exon sequencing [64] or whole-exome sequencing (WES) [62]. Trio WES analysis in multiple family members of different generations can further increase the likelihood of finding the causative mutation and can help with the interpretation of ambiguous results. A recent study showed that in approximately one-third of pediatric patients awaiting renal transplantation, a monogenic kidney disease can be identified [61], Nine of the 104 total patients (9%) were clinically categorized as having renal cystic ciliopathy, and 78% of those were molecularly diagnosed by WES [61]. Retrospective analysis of genetic studies demonstrated that the likelihood of finding the causative mutation is higher in cases with familial occurrence of renal disease, in patients with extrarenal manifestations, when there is parental consanguinity and in cases with early onset of disease [60, 61]. Homozygous deletions in the *NPHP1* gene account for approximately 21% of all NPHP cases, whereas mutations in the other genes are far less common (Table 1). Therefore, it is a reasonable approach to attempt detection of homozygous deletions in *NPHP1* as a first step, since testing is fast, cheap, and technically well established and has approximately a one in five chances of finding the causative mutation. If no *NPHP1* mutation is identified, there are two possible ways to proceed. First, one could use targeted, multiplex PCR-based screening of the next 10–15 most frequently mutated genes (gene panel sequencing [64]). This approach is fast and relatively cheap because it allows parallel sequencing of multiple patient samples in each assay. Alternatively, one could perform WES of all coding regions in the human genome ("the exome") with the advantage that in WES, the analyzed gene set is unrestricted and therefore genes that were not suspected based on the patient's clinical presentation ("phenocopies") will also be included in the analysis. Presently, cost reduction for clinically certified WES has made this option feasible for more patients. However, because in ~30% of NPHP patients mutation analysis will fail to identify a causative mutation, finding no mutation does not exclude the diagnosis of NPHP. For instance, complex genomic rearrangements cannot be detected by WES due to technical limitations. Therefore, further analysis using, e.g., genome-wide analysis of copy number variants (CNVs) [176] or whole-genome sequencing may be indicated in patients with negative test results. Presently, most of those latter techniques are used in research settings, although as research progresses the number of patients for whom we can offer a definite molecular diagnosis by genetic testing may further increase. In summary, it is logical to apply a step-wise approach for molecular diagnostics in patients with suspected NPHP-RC.

Genetic Counseling

Molecular genetic testing should only be performed with patient/parent consent within the guidelines of the National and International Societies for Human Genetics (http://www.ashg.org/press/healthprofessional.shtml). Acceptance of molecular genetic diagnostics among patients and families may increase in the United States due to the recent passing of the Genetic Information Nondiscrimination Act (GINA) by congress (http://www.ashg.org/press/ginaupdate.shtml). Before genetic counseling, a thorough pedigree analysis to distinguish recessive (early-onset) from dominant (late-onset) disease is mandatory, and extrarenal organ involvement should be interrogated. Since asymptomatic potential carriers of recessive defects should not be examined by molecular genetic diagnostics, unaffected siblings below 25 years of age should be re-evaluated yearly for urinary concentrating ability and by renal ultrasound. If symptoms evolve and serum creatinine increases, molecular genetic diagnostics following informed consent is warranted, to allow early prevention of complications such as electrolyte disturbances, dehydration, anemia, and growth retardation. If a transplant recipient's renal histology suggests NPHP and a living related donor is considered, molecular genetic diagnostics may help to exclude or detect renal disease within the potential donors. Recurrence of disease has never been reported after renal transplantation in NPHP patients. Prenatal diagnosis can be performed by direct genetic testing, if the specific mutation has already been characterized in an affected sibling.

Differential Diagnosis

On histopathology, NPHP and MCKD have to be differentiated from other forms of interstitial nephropathies like chronic pyelonephritis or drug injury. In oligomeganephronic dysplasia, kidney size is reduced, and histology is distinct from NPHP. The paucity of urinary abnormalities, the frequent lack of hypertension, normal kidney size, and the localization of renal cysts (if present) readily differentiate variants of the NPHP and MCKD from recessive or dominant polycystic kidney disease. Finally, medullary sponge kidney [177] can easily be distinguished from NPHP or MCKD, since the former does usually not lead to chronic kidney disease and demonstrates calcifications and calculi on renal ultrasound. The presence of a normal urine sediment distinguishes NPHP from monogenic proteinuric kidney diseases, such as steroid-resistant nephrotic syndrome or Alport syndrome.

Prognosis and Therapy

There are no specific therapies for NPHP. Therapy is directed toward abatement of symptoms and toward the correction of electrolyte, acid-base, and water balance disturbances, typically by a (pediatric) nephrologist. Hypokalemia may contribute to polyuria, so if detected, oral potassium supplementation may alleviate this problem. Metabolic acidosis should be corrected, and renal osteodystrophy and secondary hyperparathyroidism are treated with adequate calcium supplementation, phosphorus restriction, phosphate binders, and/or vitamin D therapy. Anemia should be treated with iron supplementation and erythropoietin, growth retardation may require administration of growth hormone, and hypertension (if present) should be treated. Adequate nutrition should be maintained with the help of a dietician. Psychological counseling of the patient and his/her parents is an integral part of therapy, because of the potential for poor self-image associated with growth retardation and chronic disease, as well as to help with the difficulties from the need to comply with complicated medications and dietary restrictions. Most patients will require renal replacement therapy by dialysis and/or renal transplantation in childhood or adolescence. Outcomes after renal transplantation are usually favorable in NPHP patients [178], and disease recurrence in the transplanted kidney has never been reported.

Despite intense research, no specific treatment for NPHP has been identified. Several experimental treatment strategies have been tested in cell culture and in in vivo models; however, none has proven to be beneficial in clinical studies to date. For example, roscovitine, a selective inhibitor of cyclin-dependent kinases, has been successfully used to mitigate the renal cystic phenotype in mouse models of NPHP-RC where the cell cycle is dysregulated [110, 179]. Furthermore, loss of function of the gene *DCDC2* was shown to cause dysregulation of the Wnt signaling pathway; in zebrafish, knockdown of the orthologous gene phenocopied the human disease, and the fish responded to treatment with a Wnt inhibitor [20]. Gattone et al. have shown that the renal cystic phenotype of *pcy* mice, which is the equivalent of human NPHP type 3, can be mitigated by treatment with the vasopressin V2 receptor antagonist OPC31260 [101]. Similar results can be found in a *pkd2* mouse model [180]. These benefits of the V2 receptor antagonists are thought to be mediated by reduction of intracellular cAMP levels [50]. In adult patients with ADPKD who fulfill specific criteria and are at risk for rapid progression, two large clinical studies demonstrated beneficial effects for the vasopressin V2 receptor antagonist tolvaptan [181, 182]. However, this treatment is not approved for patients younger than 18 years of age and has not been tested in NPHP patients; furthermore, potential benefits of tolvaptan are uncertain in these patients as the pathogenesis of NPHP differs significantly from that of ADPKD. An important future challenge will be the development of NPHP-specific therapies that capitalize on what has been learned in preclinical studies.

Cross-References

- ▶ Autosomal Dominant Polycystic Kidney Disease
- ▶ Autosomal Dominant Tubulointerstitial Kidney Disease
- ▶ Autosomal Recessive Polycystic Kidney Disease
- ▶ Assessment of Kidney Function in Children, Adolescents, and Young Adults
- ▶ Imaging Kidneys and the Urinary Tract

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Autosomal Dominant Tubulointerstitial Kidney Disease

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Abstract

Autosomal dominant tubulointerstitial kidney disease (ADTKD) is a recently defined entity characterized by tubular atrophy, interstitial fibrosis, and development of chronic kidney disease. Different forms of ADTKD can manifest in childhood; however, due to the slow progressive nature of this condition,

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renal failure usually develops in adulthood. Initially, ADTKD was known as medullary cystic kidney disease (MCKD). However, after the identification of mutations in *Uromodulin* (*UMOD*) as one of the causative genetic defects for MCKD, it became apparent that only approximately 20% of patients with *UMOD* mutations develop renal cysts and that the medulla is not a specific location for the cysts when observed. Therefore, the name "medullary cystic kidney disease" was considered misleading and contributing to underdiagnosis of this condition. Other terms used for the same condition included familial juvenile hyperuricemic nephropathy (FJHN), hereditary interstitial kidney disease, and tubulointerstitial nephritis. In order to replace these unspecific and misleading names with a more descriptive and accurate term which would also simplify diagnosis, the term "autosomal dominant tubulointerstitial kidney disease" (ADTKD) was created (Eckardt KU, Alper SL, Antignac C, Bleyer AJ, Chauveau D, Dahan K et al, Kidney Int 88:676–683, 2015). A major challenge of ADTKD is the nonspecific nature of clinical and pathological characteristics. To make the correct diagnosis, a family history is helpful and consistent with a dominant pattern of inheritance. Recently, other genes mutations (*Mucin-1, HNF1B, Renin, SEC61A1*) have also been associated with ADTKD, providing evidence that, in fact, the term ADTKD refers to a heterogeneous disease spectrum with overlapping renal phenotypes caused by at least five different genes (Devuyst O, Olinger E, Weber S, Eckardt KU, Kmoch S, Rampoldi L et al, Nat Rev Dis Primers 5(1):60, 2019). In this chapter, the genetic and clinical heterogeneity, of the pathogenesis, and potential therapeutic approaches for this condition will be discussed.

Keywords

ADTKD · Medullary cystic kidney disease · Uromodulin · Mucin-1 · HNF1B · Renin · SEC61A1

Synonyms

ADTKD - medullary cystic kidney disease; HNF1B - TCF2; UMOD - Tamm Horsfall protein

Introduction

In 1971, Bennett and Gardner first described patients with cysts of the renal medulla and named the condition medullary cystic kidney disease (MCKD) [1, 3, 4]. Over 30 years later, dominant mutations in the gene encoding Uromodulin (*UMOD*) were published in patients with MCKD type 2 [5–8]. *UMOD* mutations were also identified in patients with familial juvenile hyperuricemic nephropathy (FJHN) and glomerulocystic kidney disease (GCKD), showcasing a wide clinical spectrum caused by mutations in one gene [7]. Even prior to the discovery of *UMOD* mutations, it was noticed that the two previously named conditions MCKD2 and FJHN (which both were found later to be caused by *UMOD* mutations) displayed significant clinical overlap with polyuria, hyperuricemia, gout, tubulointerstitial fibrosis, progressive chronic kidney disease (CKD), and renal failure between 25 and 70 years of age [9]. Because overlapping phenotypes were also found with single allele mutations in *Mucin-1* (*MUC1*), *hepatocyte nuclear factor 1β* (*HNF1B*), *Renin* (*REN*), and *SEC61A1,* these conditions have now all been combined as autosomal dominant tubulointerstitial kidney disease (ADTKD) with a subclassification based on the underlying gene mutation [1, 10–13] (Table 1). Most of these genes when mutated either accumulate in renal tubular cells due to protein misfolding (e.g., UMOD, MUC1, REN) or participate in the translocation process of proteins to the endoplasmic reticulum (ER) (e.g., SEC61A1) [2]. In the ER, proteins undergo multiple modifications such as removal of the signal peptide, *N*-glycosylation, disulfide bond formation, and protein folding via oxidoreductase and chaperones. Correctly folded and modified, proteins leave the ER and then enter the Golgi apparatus and the secretory pathway. If this process is perturbed, ER stress and the unfolded protein response (UPR) are triggered, resulting eventually in cellular apoptosis and

Table 1 Clinical, laboratory, and pathological findings for the different forms of ADTKD

Subclassification	ADTKD-*UMOD*	ADTKD-*MUC1*	ADTKD-*REN*	ADTKD-*HNF1B*	ADTKD-*SEC61A1*
OMIM ID	603860/191845	174000/158340	613092/179820	614227/189907	617056/609213
Chromosome	16q12	1q22	1q32	17q12	3q21.3
Tissue expression	Kidney, TAL, and DCT	Secretory epithelia (lungs, stomach, intestine, and kidney)	Kidney (juxtaglomerular apparatus)	Kidney, pancreas, liver, lung, intestine, and urogenital tract	Ubiquitous
Protein function	Regulates ion channel transport, BP, and urinary concentration Protects against kidney stones and UTIs Regulation of innate immunity	Protection of epithelial barrier Immunomodulation Signal transduction	Protease, cleaves Angiotensinogen Role in renal development BP regulation	Transcription factor Involved in organ development (kidney, genital tract, pancreas, liver, lung, and gut)	Component of SEC61 channel-forming complex, mediates, and transport of protein precursors across the ER
Clinical	Early onset of gout Slow progressive CKD	No specific findings Slow progressive CKD	Mild hypotension, Risk for AKI↑ Slow progressive CKD Anemia	Polyuria-polydipsia CKD in childhood in severe forms Early onset diabetes mellitus Genital abnormalities Autism spectrum disorder, developmental, learning, and psychiatric problems in patients with 17q12 rearrangement	Growth retardation, neutropenia, recurrent skin infections, congenital anemia, small, dysplastic, and possibly cystic kidneys
Imaging	Occasional renal cysts	Occasional renal cysts	Normal to small kidneys without cysts	Pre- and postnatal increased renal echogenicity Renal cysts Pancreatic hypoplasia Extrarenal cysts (liver, pancreas))	Small, dysplastic kidneys without cysts
Onset in childhood	Rare	Rare	Common	Common	Common
Laboratory findings	Uric acid ↑ FEUrat <5%		Uric acid ↑ Hgb ↓ K^+↑	Uric acid ↑ Mg^{2+}↓ K^+ ↓ Abnormal LFTs	Anemia Neutropenia Growth retardation cleft palate, bifid uvula, and velo-pharyngeal insufficiency

(continued)

Table 1 (continued)

Subclassification	ADTKD-*UMOD*	ADTKD-*MUC1*	ADTKD-*REN*	ADTKD-*HNF1B*	ADTKD-*SEC61A1*
					Preaxial polydactyly Mild cognitive impairment
Histology	Intracellular accumulation of UMOD in TAL	Intracellular accumulation of MUC1-fs in distal nephron	Impaired renin staining in cells of the juxtaglomerular apparatus	Small-normal kidneys Dysplastic kidneys Glomerulocystic kidney disease	Multiple small foci of tubulointerstitial lesions and a glomerulocystic pattern
Previously used terminology	Medullary cystic kidney disease type 2 Uromodulin-associated kidney disease Familial juvenile hyperuricemic nephropathy Uromodulin kidney disease Hereditary interstitial kidney disease Tubulointerstitial nephritis Glomerulocystic kidney disease	Medullary cystic kidney disease type 1 Mucin-1 kidney disease Hereditary interstitial kidney disease Tubulointerstitial nephritis	Familial juvenile hyperuricemic nephropathy Hereditary interstitial kidney disease Tubulointerstitial nephritis	MODY5 (maturity onset diabetes mellitus of the young type 5) RCAD (renal cyst and diabetes syndrome) Familial juvenile hyperuricemic nephropathy Hereditary interstitial kidney disease Tubulointerstitial nephritis	N/A

AKI acute kidney injury, *BP* blood pressure, *DCT* distal convoluted tubule, *ER* endoplasmic reticulum, *FEUrat* fractional excretion of uric acid, *HNF1B* hepatocyte nuclear factor 1B, K^+ potassium, *LFT* liver function test, Mg^{2+} magnesium, *MUC1* Mucin-1, *REN* Renin, *SEC61A1* α1 subunit of transport protein SEC61, *TAL* thick ascending limb, *UMOD* Uromodulin organ failure. Due to the histopathological similarities between ADTKD and nephronophthisis, these conditions are sometimes also described by pathologists as a dominant version of nephronophthisis. Whereas UMOD is mostly expressed in tubular cells of the thick-ascending limb of Henle's, the other gene products are mostly expressed in the intermediate and distal nephron. Typically, mutations in *UMOD*, *MUC1*, and *REN* are confined to renal defects, although mutations in *HNF1B* and *SEC61A1* frequently lead to extrarenal manifestations (Table 1) [1].

Epidemiology of ADTKD

Given the rarity of ADTKD and the likely underdiagnosis of the condition, it is difficult to provide accurate epidemiological data. Presently, there are no obvious differences in ADTKD prevalence based on gender, nationality, or ethnicity. It is likely that ADTKD is disproportionately underdiagnosed in African-Americans given their overall reduced access to medical care and that they have other frequent causes of CKD such as *APOL1*-related kidney disease [14]. Among the different causes of ADTKD, it appears that mutations in *UMOD* and *MUC1* represent the most common forms of the disease. Initially, the prevalence of ADTKD-*UMOD* was reported as 1.67 cases per million [1, 15]. However, a recent study that identified *UMOD* mutations in five kidney transplant recipients among a total of 911 patients suggests that ADTKD-*UMOD* is more common than originally suspected [16]. In a recent CKD patient cohort, about 9% were found to have a monogenetic disease and 3% had a *UMOD* mutation, accounting for about 0.3% of all CKD patients in this group [17]. Together, among the estimated 37 million CKD patients in the United States, there may be approximately 1.1 million patients with a *UMOD* mutation. In an Irish cohort, 2.6% of CKD patients had a *UMOD* mutation [18]. In a separate Irish cohort, mutations in *UMOD*, *MUC1*, and *HNF1B* were found in 0.54% of patients with renal failure [19]. In an English CKD cohort, it was estimated that ADTKD has a prevalence of 16 per million with an ADTKD-*UMOD* prevalence of 9 per million (representing approximately 1% of patients with CKD stages 3–5) [20]; thus, in this population, ADTKD may be the most common monogenetic kidney disease after ADPKD and collagen IV mutations. In the United States, ADTKD-*MUC1* mutations are estimated to have a prevalence of 0.7 per million individuals, while in Ireland a prevalence of 4 per million has been estimated [2]. Together, these data suggest that ADTKD, and in particular ADTKD-*UMOD,* is fairly common and still underdiagnosed, due to a lack of specific clinical characteristics. The prevalence of *HNF1B* point mutations and deletions in the general population is unknown. It is likely that many cases are incorrectly diagnosed due to the variable phenotype, incomplete gene penetrance, and the high frequency of *de novo HNF1B* deletions. Among patients with structural kidney abnormalities, the *HNF1B* mutation rate varies between 5% and 31% and constitutes the most frequent cause of kidney dysplasia of monogenic origin in pediatric cohorts. Diagnosis in children can often lead to diagnosis that was unrecognized in affected family members [11, 21]. Among the causes of ADTKD, mutations in *REN* and *SEC61A1* are much less common with about 30 identified ADTKD-*REN* families and only two ADTKD-*SEC61A* families [12, 13, 22].

Presentation of ADTKD

Clinical Characteristics

Given the new unifying terminology, an increasing number of ADTKD cases have been published, suggesting that ADTKD is one of the more common monogenic kidney diseases [17, 20, 23]. A main reason that ADTKD has been overlooked and underdiagnosed is that clinical, laboratory, and pathological findings are nonspecific. To make the correct diagnosis, it helps to look at the holistic presentation in the context of all clinical findings. Common features of ADTKD are progressive CKD, absent-to-mild proteinuria, normal or mildly elevated blood pressure, and normal (early diagnosis) to small (later diagnosis) kidneys (Table 2). The most prominent feature of

Table 2 Clinical findings in ADTKD patients

Autosomal dominant inheritance
Progressive worsening of chronic kidney disease
Unremarkable urine sediment
No proteinuria or only mild proteinuria
During the early stages no severe hypertension
Early-onset hyperuricemia or gout
Enuresis or nocturia in children
Urinary concentration defect, polydipsia-polyuria in severe forms
Early chronic kidney disease in severe forms in children
Renal ultrasound shows normal or small-sized kidneys
Lack of exposure to medications causing tubulointerstitial nephritis

ADTKD is the autosomal dominant inheritance pattern (Table 2). However, the absence of a family history does not rule out the condition, as *de novo* mutations may occur, in particular for ADTKD caused by *HNF1B* mutations [24]. A few cases of ADTKD have had two mutations in *UMOD* in the same individual, leading to a more severe phenotype [25]. Penetrance within families can be variable, although disease usually appears in all affected family members if they live long enough. Disease severity and age of onset are quite variable even within a family that has the same mutation. For certain genetic subcategories of ADTKD, extrarenal manifestations may be prominent. For example, *HNF1B* mutations commonly present with a range of extrarenal symptoms, and rarely the tubulointerstitial kidney disease is the sole manifestation [26, 27]. Therefore, the term ADTKD-*HNF1B* should be reserved for patients with *HNF1B* mutations in which the tubulointerstitial kidney disease is the prominent presentation [1]. Mutations in *SEC61A1*, which encodes the α1 subunit of the SEC61 translocon pore, usually result in more syndromic forms of kidney disease with intrauterine or postnatal growth retardation, neutropenia (causing recurrent skin infections), congenital anemia, and small, dysplastic kidneys which may contain cysts [13]. Age of onset is variable and often dependent on the ADTKD subtype. Whereas ADTKD-*UMOD* patients may present with gout and CKD in the late teen years, ADTKD-*MUC1* patients usually present with CKD (only) in the early 20s. ADTKD-*HNF1B* may be present in fetuses or infants as the most common cause for increased bilateral renal echogenicity. Genitourinary abnormalities are often found in childhood, and gout can present in the teenage years in these patients [27, 28]. Maturity-onset diabetes of the young (MODY), associated with *HNF1B* mutations, usually does not present until the third decade of life. Studies have shown that in children with *HNF1B* mutations, kidney disease led the clinical presentation, whereas in adults MODY-type-diabetes-related symptoms predominated [28–30]. Finally, ADTKD-*REN* can present in early infancy with hyperkalemia, CKD, and anemia.

Chronic Kidney Disease

The rate of decline of renal function is quite variable, often dependent on the genetic mutation causing ADTKD [24, 26, 31]. The age of onset for renal replacement therapy varies significantly among and within families with ranges between the ages of 20 years and 80 years. Most ADTKD patients require initiation of renal replacement therapy between 30 and 50 years of age. In rare cases, renal replacement therapy is required in childhood [32]. CKD can present in childhood with smaller kidneys, increased echogenicity, hyperuricemia, or gout [32–38]. Creatinine may be only mildly elevated. While end stage kidney disease due to ADTKD typically occurs after 30 years of age, it can occur in childhood and has been described as early as 6–7 years of age with *UMOD* and *HNF1B* mutations [23, 26, 37]. ADTKD-*MUC1* patients typically develop end stage kidney disease earlier than ADTKD-*UMOD* patients (46 vs. 54 years, respectively) [39]. While ADTKD-*HNF1B* usually causes renal failure between the age of 25 and 70, infants with *HNF1B* mutations and severe kidney hypoplasia-dysplasia may develop end stage disease early in life. As REN regulates renal fluid and electrolyte absorption, patients with *REN* mutations are at higher risks for volume depletion and hypotension, which may aggravate the risk for CKD.

Urinary Concentration Defect and Enuresis

Certain forms of ADTKD result in urinary concentration defects and enuresis. In a large family with ADTKD-*UMOD,* the maximum urine osmolality was 593 ± 76 mOsm/kg compared to 918 ± 239 mOsm/kg in unaffected individuals [40]. In the same ADTKD-*UMOD* kindred, 10 out 39 family members had enuresis [40]. The urinary concentration defect is thought to stem from accumulation of mutant UMOD in the thick ascending limb of Henle (TAL) which disturbs TAL function and leads to polyuria [41]. The urinary concentration defect has also been described in animal models of ADTKD-*UMOD* [42]. As a consequence of the chronic volume depletion, the proximal tubule increases fluid absorption in a compensatory fashion and absorbs more uric acid in parallel which contributes to the hyperuricemia associated with ADTKD.

Hyperuricemia and Gout

UMOD and *HNF1B* mutations can result in early onset of gout as early as 3 years of age. A dominant family history of gout is present in up to 75% of male and 50% of female ADTKD patients and suggests the possibility of *UMOD* or *HNF1B* mutations [24]. In male ADTKD patients, gout can present early in life (range 3–51 years) [24, 37]. ADTKD-*UMOD* patients typically present much earlier with gout than ADTKD-*MUC1* patients (30 vs. 67 years, respectively) [39]. Frequently, gout presents in childhood prior to the onset of significant renal impairment. While hyperuricemia and gout may lead to the diagnosis, both of them are not a diagnostic requirement for ADTKD. In fact, hyperuricemia and gout may occur after the onset of severe CKD [24].

Hypomagnesemia

Refractory hypomagnesemia of renal origin has been described with various types of *HNF1B* mutations [43]. As the hypermagnesuria in these patients is usually not associated with hypercalciuria, the impairment of renal Magnesium (Mg^{2+}) reabsorption occurs in the distal nephron and not the TAL. In the distal convoluted tubule, HNF1B binds to the promoter of the *FXYD2* gene which encodes the γ subunit of the Na^+/K^+-ATPase; mutations in *FXYD2* cause autosomal dominant hypomagnesemia, and HNF1B is thought to regulate *FXYD2* [44]. While hypomagnesemia can be observed in children, the frequency of hypomagnesemia in patients with *HNF1B* mutations increases with age and is predominant after the second decade of life [43, 45, 46]. In adults with *HNF1B* mutations, hypokalemia has been frequently observed [25].

While hypomagnesemia is not a common problem in ADTKD-*UMOD* patients, deletion of UMOD in mice also resulted in hypermagnesuria and hypomagnesemia [47]. UMOD increases the cell surface abundance of the apical Mg^{2+} channel TRPM6. Urinary UMOD levels are higher in hypomagnesemic mice suggesting a feedback mechanism where UMOD enhances TRPM6 activity in a Mg^{2+}-deficient state. Why magnesium levels are normal in most patients with *UMOD* mutations is unknown.

Hypertension

Most ADTKD patients do not present with hypertension; however, with more advanced CKD, hypertension is common [40].

Anemia

Anemia is typically found in patients with *REN* mutations and is unrelated to the degree of GFR impairment [12]. The cause for the anemia is not well understood. It is thought that Renin-producing cells may have the capability to secrete erythropoietin and that loss of REN function leads to anemia [48]. The anemia in ADTKD-*REN* patients usually resolves during puberty, correlating with the rise of testosterone.

Imaging

In most forms of ADTKD, kidney ultrasound studies are nonspecific and nondiagnostic. In most patients, kidney size is initially normal. As ADTKD progresses, kidney size declines and cysts can occur in varying numbers and sizes [2]. Overall, the frequency of cysts in ADTKD is not thought to be more common than in other “non-cystic” forms of chronic kidney disease [24, 49]. In some ADTKD patients, glomerular cysts have been described [7, 36]. In ADTKD-*HNF1B* patients, large, hyperechogenic kidneys may be present prenatally [28]. Bilateral cystic kidney dysplasia occurs in 20–30% of cases caused by *HNF1B* mutations [29]. Renal dysplasia and glomerulocystic kidney disease may also present in fetuses with ADTKD-*HNF1B* nephropathy [50].

Laboratory Studies

The urinary sediment is usually normal or may reveal microhematuria. Proteinuria is absent or mild [24, 26, 31]. *UMOD*, *REN*, and *HNF1B* mutations can cause hyperuricemia. Elevated uric acid levels, even early in childhood, tend to suggest these forms of ADTKD. Most of these patients have an abnormally low fractional excretion of uric acid ($Fe_{Urat} < 5\%$, normal FE_{Urat} is $>5\%$). However, Fe_{Urat} is frequently reduced in any type of CKD with a GFR < 80 ml/min/1.73 m^2 and, therefore, is not a reliable diagnostic criteria for ADTKD [24, 41]. Changes in Renin and Aldosterone levels are not diagnostic for ADTKD-*REN*. Studies have found lower urinary secretion of UMOD in ADTKD-*UMOD* patients, although this test is not readily clinically available [6, 32, 38].

Genetic Testing

The gold standard for diagnosis of ADTKD is identification of a causative mutation. Relatives of an affected individual have a 50% likelihood to have the mutation. Genetic testing is recommended in adults in whom ADTKD is suspected, which will inform the ability to donate a kidney and/or assist in prepregnancy planning [2]. However, given the lack of specific therapeutic options to treat ADTKD at this point, testing of unaffected minors is discouraged [51]. Many practitioners think that the choice of testing with the potential psychologic burden with a disease diagnosis with no specific therapy is best left to the child once they come of age. A quality of life survey in adults with ADTKD showed that “ADTKD has a substantial effect” on their life; 23% of diagnosed patients thought about it daily, 26% weekly, 26% monthly, and 26% less than monthly [52]. While anxiety was similar between ADTKD-affected and control individuals, the affected patients were more frequently depressed. Therefore, it may be appropriate to leave the question of genetic testing to the individual once they are old enough to decide for themselves.

The decision about whether to do genetic testing is difficult and complex and should be openly discussed with the family. Many families are thankful for a diagnosis even without a specific therapy as it often resolves a long search for a cause of their disease. Among a group of 55 individuals who were genetically diagnosed with ADTKD with no foreknowledge about their renal disease, 93% were happy that testing was performed, 5% remained neutral, and only 2% were unsatisfied [52]. A definitive diagnosis prevents the need for further diagnostic tests which reduces further cost and time expenditure. Additionally, genetic testing may be considered in families with a high prevalence of gout as therapy with allopurinol or febuxistat may prevent gout [2]. Furthermore, a definitive diagnosis can provide benefits in children with ADTKD-*REN* or ADTKD-*HNF1B*. In the case of ADTKD-*REN,* one can screen for anemia and treat with erythropoietin if needed. Hypotension and hyperkalemia could be treated with agents such as Fludrocortisone. Patients could be counseled on appropriate precautions to avoid volume contraction and to avoid NSAID use to decrease risks of AKI. In ADTKD-*HNF1B*, hyperuricemia, which presents in about a third of patients, can be treated with allopurinol or febuxistat. These patients can be also screened for hypomagnesemia and hyperglycemia. Also, postkidney transplant immunosuppressive management can be modified to reduce the risk of new-onset diabetes mellitus after transplantation (NODAT), which is more prevalent in ADTKD-*HNF1B* patients [53]. As ADTKD-*HNF1B* patients have a high likelihood of underlying hypomagnesemia, use of tacrolimus is discouraged as it may exacerbate hypomagnesemia [43]. Hypomagnesemia and steroid exposure may trigger development of NODAT, which reduces survival of the kidney allograft [54–56]. Parents and possible candidates for testing (dependent on age) should be involved in a discussion about costs, test interpretation, and future implications. In the United States, an ADTKD diagnosis may affect future health and/or life insurance coverage. As new ADTKD treatments become available, early genetic diagnosis may become the goal to initiate this therapy as early as possible. As ESKD often happens later in life, pediatric nephrologists may be critical in providing these newer therapies.

Preclinical studies have shown promise for future therapeutic strategies [57].

Genetic testing for *UMOD*, *REN*, and *HNF1B* is well established in clinical practice and can be performed by commercial genetic testing laboratories. *HNF1B* mutations can consist of large deletions requiring a multiplex ligation-dependent probe amplification (MLPA) assay to avoid missing the mutation by sequencing of the *HNF1B* gene. Identifying *MUC1* mutations may be challenging. *MUC1* mutations are usually a single base pair insertion within the variable nucleotide tandem repeat (VNTR) which consists of 60 base pair motifs encoding for 20 amino acids. *MUC1* has 25–125 VNTR repeats translating into 1.5 and 7.5 kb of sequence. Moreover, the VNTR is a highly GC-rich region, and as the insertion occurs variably within the VNTR for each family, detecting *MUC1* mutations is highly challenging [10]. Only very few laboratories including the Broad Institute in Boston are capable of reliably performing MUC1-genetic testing (https://www.broadinstitute.org/rare-diseases/muc1-kidney-disease-mkd). Different diagnostic tools for identifying *MUC1* mutations/insertions in the VNTR include SNaPshot minisequencing, single molecule real-time sequencing, and immunostaining using antibodies that detect mucin 1 neoproteins (MUC1-fs) in kidney biopsies and urinary cells [58–60]. While none of these assays have become routine testing yet, the noninvasive detection of MUC1-fs in urinary cells may be the most useful diagnostic tool in the future [60]. Despite advances in molecular diagnostics, mutations in *UMOD*, *MUC1*, *REN*, and *HNF1B* are only responsible for 50–60% of all ADTKD cases, indicating that mutations in other not yet identified genes may cause ADTKD [2]. In a recent study of an ADTKD cohort of 726 patients, *UMOD* mutations were the most common identified in 38.4% followed by MUC1 mutations in 35.1% [39].

Pathology Findings

Renal biopsies for ADTKD show interstitial fibrosis, tubular atrophy, and thickened glomerular basement membranes [9, 24, 26, 49] (Fig. 1a). Lamellation and thickening of tubular basement membranes and tubular dilatation are typically observed [6, 9]. These findings help to differentiate ADTKD from tubulointerstitial fibrosis observed with other types of progressive CKD that occurs due to matrix accumulation from tubular cells [61, 62]. Renal cysts can be identified although they are not considered to be more commonly seen than in other “non-cystic” forms of chronic kidney disease [24, 26, 31, 49]. As cysts are more frequently found in patients with more advanced ADTKD than those with early stages of disease, cysts are not likely to be causing the decline in renal function [1]. Glomeruli are usually not involved, although rare patients may have secondary focal and segmental glomerulosclerosis or with glomerulocystic kidney disease [7, 36]. Immunofluorescent studies for complement and immunoglobulins may exclude other causes of CKD but cannot be used to directly diagnose ADTKD. Intracellular cytoplasmic inclusions of lamellated aggregates derived from mutant proteins may be detected (Fig. 1b). Immunostaining for UMOD or MUC1-fs may reveal focal, coarse, and dense accumulation of mutant protein aggregates [10, 63] (Fig. 1c, d). The intracellular mutant protein accumulation in ADTKD-*UMOD* explains the lower urinary UMOD concentration seen in these patients [6, 32, 38]. The ability to detect lower urinary and/or plasma levels of UMOD and to detect urinary MUC1-fs offers the possibility for future noninvasive-testing strategies. Electron microscopy imaging may show fibrillar material in expanded stacks of ER of the thick ascending limb, most likely representing accumulation of mutant UMOD protein [63, 64] (Fig. 1e, f).

Differential Diagnosis

Infrequently, nephronophthisis (NPHP) may present in young adults or later in life [65–67] and as such may be misidentified as ADTKD. The recessive inheritance pattern with NPHP should help to distinguish NPHP from ADTKD, except in rare cases of pseudodominant inheritance of NPHP [65, 68]. Patients with ADPKD typically have much larger kidneys and more prominent cyst development than ADTKD. Biopsy findings in

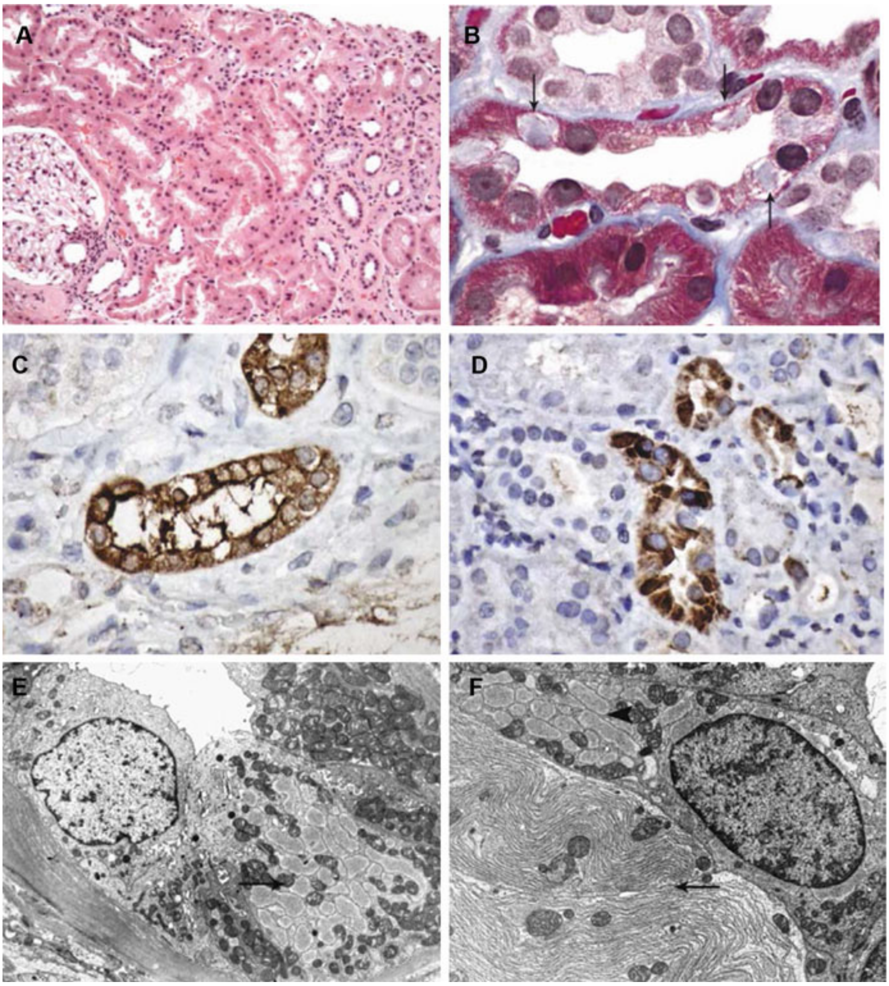

Fig. 1 **Renal histology of ADTKD-*UMOD*.** (**a**). At lower magnification, the renal cortex of an ADTKD-*UMOD* patient shows focal patchy tubular atrophy, interstitial fibrosis, and mild interstitial inflammation. No tubulitis is observed (Hematoxylin and eosin, original magnification x200). (**b**) At higher magnification, focal intracytoplasmic inclusions of lamellated trichrome blue material (arrows) are seen which contrast with the red-stained cytoplasm (Masson trichrome, original magnification x600). (**c**) In a control kidney, UMOD is detected in a homogenous, diffuse pattern in the cytoplasm and stronger along apical membranes (original magnification x600). (**d**) In an ADTKD-*UMOD* patient, focal, coarse, dense clusters of mutant UMOD accumulate within the thick ascending limb (original magnification x600). (**e**) Electron microscopic imaging showed dilated cisternae of the ER with accumulation of granular, electro-dense storage material. The tubular membrane is thickened and lamellated (Electron micrograph, original magnification x4000). (**f**) Electron microscopic imaging of a thick ascending limb cell displays hyperplastic bundles of the ER (arrow) with few intervening mitochondria and areas of dilated ER that contain elevated levels of material (Electron micrograph, original magnification x4000). All figure panels were published in [63]

ADTKD may resemble other forms of tubulointerstitial kidney disease caused by autoimmune processes, medication toxicity (NSAIDs, calcineurin inhibitors, and lithium), or tubulointerstitial nephritis and uveitis syndrome [2]. Extrarenal manifestations are rare in ADTKD except with *HNF1B,* and *SEC61A1* mutations. In children, congenital abnormalities of kidney and urinary tract

(CAKUT) of other origin are in the differential diagnosis. Presence of normal amniotic fluid, bilateral normal-small sized kidneys, often with cysts and extrarenal involvement with ADTKD, may help to differentiate between the former and other causes of CAKUT [27–30, 45]. That said, ADTKD-*HNF1B* patients may present with oligo-/anhydramnios and/or massively enlarged and/or cystic kidneys that mimic autosomal recessive polycystic kidney disease (ARPKD) or early ADPKD. These phenotypes may occur as HNF1B is a master regulator of *PKHD1* and *PKD2* genes [69]. Genetic testing can help clarify the diagnosis [70]. Atypical forms of ADPKD-ADTKD due to heterozygous *DNAJB11* mutations (with normal-sized kidneys, kidney fibrosis, slowly progressing CKD, and high prevalence of renal cysts) should be considered in patients with no mutations in *UMOD*, *MUC1*, *REN*, *HNF1B*, *or SEC61A1* [71]. A one allele loss of *DNAJB11* perturbs maturation and trafficking of polycystin-1 and UMOD, with retention of UMOD in the TAL, similar to ADTKD-*UMOD* [71]. Homozygous *DNAJB11* mutations result in Ivemark II syndrome, with oligohydramnios and severe renal-hepatic-pancreatic dysplasia [72]. Renal histology with the latter syndrome also reveals interstitial fibrosis and multiple renal cysts. Mutations in *PAX2* and mitochondrial DNA can also result in tubulointerstitial kidney disease similar to what is seen in ADTKD [73, 74]. The ocular findings in patients with renal coloboma syndrome (caused by *PAX2* mutations), if present, should differentiate these patients from ADTKD [75]. In mitochondrial diseases, only maternal transmission occurs and as such may differentiate the former from ADTKD. Other causes of hyperuricemia such as Lesch-Nyhan (caused by X-linked *HPRGT* mutations) are usually accompanied by more severe developmental and behavioral problems such as self-mutilation and choreoathetosis than in ADTKD patients [76]. Patients with hyperuricemia and kidney stones but normal neurologic development may have Kelley-Seegmiller syndrome which is a milder form of Lesch-Nyhan syndrome, caused by only partial inactivation of the *HPRGT* gene [77].

Specific Forms of ADTKD

ADTKD-UMOD Is Caused by Mutations in Uromodulin

For over 140 years, Uromodulin (UMOD), also called Tamm-Horsfall protein, has been known to be the most abundant protein in human urine [78]. The Italian physician Carlo Rovida first described UMOD as a product of renal tubular cells forming hyaline casts in the tubular lumen in 1873 [78]. In 1950, Igor Tamm and Frank Horsfall described an abundant urinary mucoprotein which inhibited viral hemagglutination and was subsequently named Tamm-Horsfall protein [79]. In 1985, a 85 kDa glycoprotein was isolated from the urine of pregnant women and was found to inhibit human T-cells and monocyte activity. Therefore, the protein was named Uromodulin [80]. The gene *UMOD* (OMIM 191845) is located on chromosome 16p12.3 and encodes the 85 kDa glycoprotein Uromodulin (UMOD) which is exclusively synthesized in the kidney where it is expressed within the TAL and the early distal convoluted tubule [5, 80–86] (Fig. 2a). UMOD is the major constituent of hyaline casts. UMOD has a high carbohydrate content (30%) and many cysteine residues and forms large protein aggregates [87]. In 1987, investigators reported that Tamm-Horsfall protein and UMOD are the same protein [81]. UMOD is synthesized as a precursor of 640 amino acids (Fig. 2b). It contains a signal peptide encoded by 24 amino acids which targets the nascent protein to the ER and is cleaved during protein maturation. The mature urinary protein is 616 amino acids long and consists of 48 cysteine residues which form 24 disulfide bridges [81, 88]. The signal peptide is followed by four epidermal growth factor (EGF)-like domains, a cysteine-rich domain of unknown function (called D8C domain), and a bipartite C-terminal Zona Pellucida (ZP) domain (Fig. 2b). Whereas the EGF-like domains are thought to be important for protein-protein interaction, the ZP domain is crucial for protein assembly into larger, extracellular, supramolecular polymers (Fig. 2c, d)

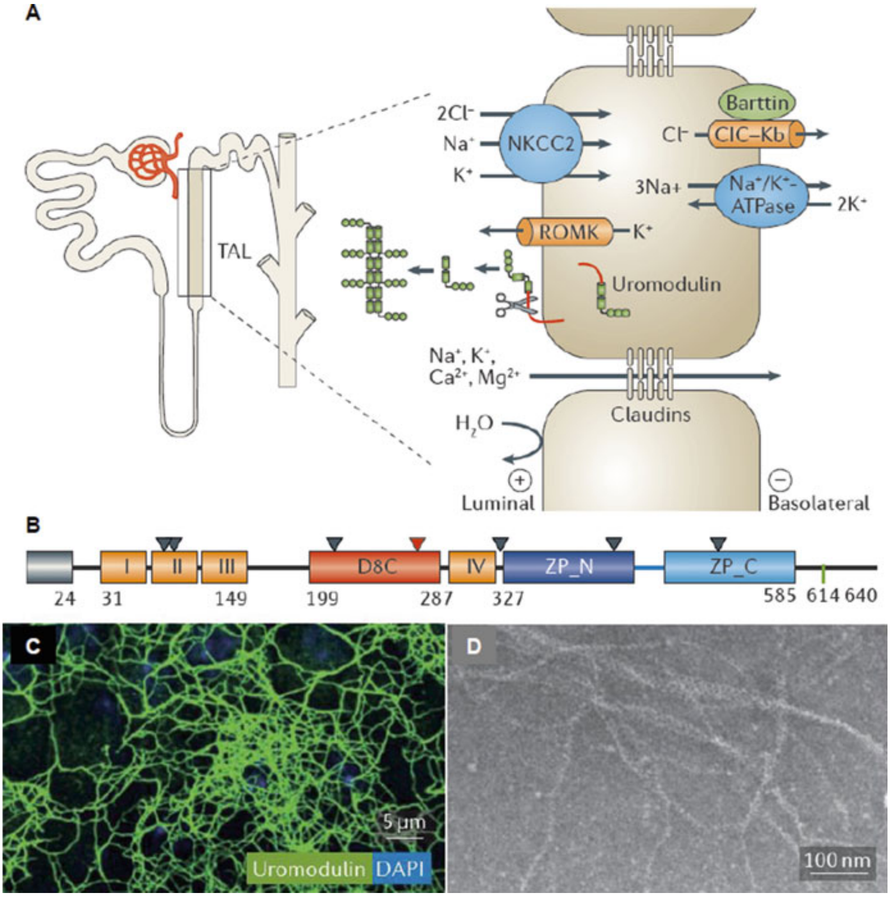

Fig. 2 **UMOD protein sequence, expression, and function**. (**a**) UMOD is synthesized in the thick ascending limb and the distal convoluted tubule. UMOD is targeted to the apical membrane where it is cleaved and released into urine by the serine protease hepsin. In urine, UMOD forms polymers and enhances NKCC2 and ROMK activity in the TAL. Recycling of K^+ via ROMK contributes to the lumen-positive transepithelial voltage which is the major driving force for paracellular Ca^{2+} and Mg^{2+} absorption. (**b**) The protein structure of UMOD includes a signal peptide (grey), four EGF-like domains (orange) which are important for protein-protein interaction, a cysteine-rich domain of unknown function (red), and a bipartite Zona Pellucida domain (ZP-N and ZP-C shown in different shades of blue). Position 614 (green) is a glycosylphosphatidylinositol-anchoring site. The seven *N*-glycosylation sites are indicated by arrowheads. A high mannose chain on residue Asn275 is identified by a red arrowhead. (**c**) Immunofluorescence imaging shows a network of UMOD filaments (green) covering a monolayer of primary TAL cells from a microdissected mouse kidney. (**d**) A transmission electron microscope image displays UMOD polymers secreted by primary mouse TAL cells. All figure panels were published in [78]

[89]. In addition to apical expression of UMOD and urinary secretion, UMOD is also thought to be released from the basolateral membrane of TAL cells into the bloodstream [83, 92], although plasma concentrations are approximately 1000-fold lower than in the urine [90–92].

Protein Function

UMOD is the most abundant protein in human urine with 50–150 mg generated per day and forms a porous 3D matrix (Fig. 2c, d) [93, 94]. UMOD plays a role in renal development and is expressed in the kidney between the 8th and 16th week of gestation, increasing steadily in quantity with maturation of the TAL [95]. UMOD is synthesized in the TAL (90%) and the DCT (10%), two nephron segments with significant roles in tubular transport of sodium, potassium, calcium, magnesium, and chloride [84, 96]. UMOD protects against urinary tract infections, acute kidney injury, and kidney stone formation and contributes to water homeostasis, renal salt handling, and immunomodulation [87, 97–101]. In addition, UMOD may play a role in the water impermeability of the TAL by forming a hydrophobic, gel-like structure which covers the apical membranes [100]. Mice lacking UMOD (*Umod*$^{-/-}$) have impaired urinary concentration ability after water deprivation [102]. Studies in *Umod*$^{-/-}$ mice also revealed that UMOD protects against urinary tract infections by binding to type1-fimbriated *E. coli*. UMOD interferes with bacterial adhesion and internalization into the uroepithelium [97, 103]. UMOD was subsequently confirmed to be protective against UTIs in a large human study that showed that higher urinary UMOD levels conferred a lower risk of UTIs [104, 105]. *Umod*$^{-/-}$ mice also develop urinary Ca^{2+}-containing crystals which cause renal calcinosis, ureteral obstruction, and hydronephrosis [98, 106, 107]. UMOD is thought to prevent renal crystal formation in a complex with other urinary proteins such as osteopontin, as a polyanionic macromolecule by coating the crystal surface and preventing crystal nucleation [108, 109]. In addition, UMOD may prevent formation of Ca^{2+} crystals by increasing membrane expression of the apical renal calcium channel, transient receptor potential cation channel subfamily V member 5 (TRPV5), by interfering with the channel's endocytosis, thus blocking hypercalciuria [99]. In humans, Ca^{2+} and uric acid stone formers excrete less urinary UMOD. In control individuals, a linear correlation exists between urinary UMOD and Ca^{2+}; however, this correlation was not found in stone formers, suggesting that stone formers are unable to secrete more UMOD with higher urinary Ca^{2+} concentrations [110]. In another study, stone formers had a 32% lower urinary UMOD concentration and a lower sialic acid content than control patients suggesting a role for UMOD and/or UMOD glycosylation in prevention of nephrolithiasis [111]. A genome-wide-association-study (GWAS) also identified a single nucleotide polymorphism (SNP) within *UMOD* which was associated with protection against formation of Ca^{2+}-containing kidney stones [112]. UMOD seems to have pleiotropic effects on tubular ion channels and also affects blood pressure. Exogenous UMOD enhances Na^{+}-K^{+}-$2Cl^{-}$-cotransporter (NKCC2) and Na^{+}-Cl^{-}- cotransporter (NCC) function and causes hypertension. However, mutant UMOD interferes with NKCC2 trafficking to the apical membrane of TAL cells, thereby contributing to TAL dysfunction, a urinary concentration defect and modest volume depletion [42, 84, 101, 102, 113]. Specific SNPs in the *UMOD* gene in healthy individuals are associated with increased UMOD secretion and ion transporter activity. Mice overexpressing WT UMOD displayed a 80% higher UMOD secretion and subsequently developed hypertension and left ventricular hypertrophy due to higher phosphorylated (activated) NKCC2 [101]. A role for UMOD in blood pressure regulation has been confirmed using *Umod*$^{-/-}$ mice which displayed lower blood pressures and higher urinary Na^{+} excretion compared to wild-type animals [114]. Correlatively, a study showed that a human cohort with the SNP rs12917707 (G/G allele) in *UMOD* resulted in reduced kidney function and left atrial remodeling [115]. UMOD also modifies ion channels such as the apical potassium and magnesium channels ROMK and TRPM6, respectively [47, 116]. Urinary and plasma UMOD levels correlate with renal mass and function and have been utilized as early biomarkers and predictors of acute kidney injury [90, 117–120]. A lower spot urine UMOD/creatinine ratio prior to cardiothoracic surgeries correlated with a higher risk of more severe AKI after the procedures [119, 120]. *Umod*$^{-/-}$ mice displayed delayed recovery after ischemic injury [121]. Lower urinary and serum UMOD levels have also been observed in patients with early kidney allograft failure [122, 123]. An association between urinary UMOD secretion and estimated glomerular filtration rate (eGFR) has been postulated [91, 117, 124]. Urinary UMOD likely reflects tubular activity with 24 h-urinary-UMOD levels being directly proportional to urine volume and kidney size [125]. Genetic variants near the *UMOD* gene are associated with higher renal UMOD expression, hypertension, eGFR, and/or decline of renal function [125–133]. Plasma UMOD levels correlate with creatinine clearance in CKD patients and could potentially help to track progression of disease in CKD patients [90–92]. Even in children with non-ADTKD CKD, serum UMOD levels were reliable indicators for CKD progression [134, 135].

Outside of ADTKD, common variants in *UMOD* are associated with higher risks of other types of CKD and end stage kidney disease [126, 127, 133]. Genome-wide association studies identified *UMOD* promoter and *UMOD* variants associated with declines in renal function and risks of CKD in the general population [112, 126, 128, 133, 136, 137]. The *UMOD* variant rs13333226 was found to be associated with increased risks of diabetic nephropathy [138]. Other *UMOD* variants were found to increase urinary UMOD expression which correlated with salt-sensitive hypertension and kidney damage [101, 125]. Another *UMOD* variant, rs4293393, also results in increased urinary UMOD levels but is associated with a lower risk of UTIs [105]. It is possible that high frequency of this ancestral latter UMOD allele (which may lead to hypertension and kidney damage later in life) protects against UTIs during the earlier years and thereby improves fitness and reproduction. There are conflicting data whether high urinary UMOD has a protective effect or aggravates chronic kidney disease. While some contend that urinary UMOD increases tubular salt absorption and thus may worsen hypertension, others suggest that UMOD bolsters immunity. UMOD modulates the innate immune system by binding of cytokines, monocytes, dendritic cells, and immunoglobulins via Toll-like receptor 4 (TLR4) [121, 139–143], and as such, UMOD deficiency causes a state of inflammation [141]. UMOD also modifies granulopoiesis and enhances macrophage phagocytic activity in response to AKI [139]. UMOD promotes transition from the proinflammatory to the healing stage during recovery. Cross-talk from the TAL to the proximal tubule and the TAL has been proposed with UMOD-inhibiting chemokine signaling and thereby reducing damage to the neighboring proximal tubule [121, 144]. UMOD also appears to have pleiotropic effects on metabolism and longevity. In a cohort of patients with coronary artery disease, higher serum UMOD levels correlated with decreased mortality [145]. In a follow-up study, higher serum UMOD levels were associated with a favorable metabolic profile with lower rates of hypertension, diabetes mellitus, heart failure, and 10-year mortality [146]. Lower serum UMOD levels also correlate inversely with HgbA1c and serum glucose values [138, 146–148]. Lower urinary and serum UMOD levels were also identified in patients with type 1 and type 2 diabetes mellitus and diabetic nephropathy [149–155]. It remains unclear if and how higher serum UMOD concentrations exert a protective effect on metabolism whereas higher urinary UMOD are associated with higher rates of hypertension and chronic kidney disease. One study showed that systemic UMOD inhibits oxidative stress by inactivation of the TRPM2 channel, and thus, serum UMOD could provide some systemic protection [156]. Together, UMOD function in urine and serum could be different, accounting for the differences in apparent protective versus deleterious effects of UMOD.

Recent data have begun to identify how tubular UMOD secretion is modified. One study found that the Ca^{2+} sensing receptor (CaSR) reduces UMOD secretion [157]. Using mouse models, *Casr*-activating mutations decreased urinary UMOD secretion, whereas inactivating mutations increased UMOD secretion. Humans treated with cinacalcet also had lower urinary UMOD secretion [157]. Additionally, hypomagnesemia increased urinary UMOD secretion in mice [47]. Moreover, high dietary NaCl intake increased renal UMOD mRNA and protein expression in rats [158]. Other studies suggest that urinary secretion of UMOD may also be salt sensitive in humans [131, 159]. Finally, high protein intake increases UMOD secretion while antidiuretic and thyroid hormone reduce UMOD secretion [93, 160, 161]. HNF1B binds to specific *Umod* chromatin DNA target sites and increases mouse UMOD expression. Mice lacking *Hnf1b* have significantly reduced urinary UMOD secretion [69].

Clinical Presentation

Based on the inverse relationship between urine osmolality and uric acid levels, ADTKD-*UMOD* patients are thought to have a urinary concentration defect and mild salt-wasting phenotype [41]. Children with ADTKD-*UMOD* also have a higher incidence of enuresis than the general population [40]. About 25–87% of patients with ADTKD-*UMOD* present with hyperuricemia, gout, and a low $FE_{Urat} < 5\%$ [24, 162]. A family history of gout is very common in these patients and is attributed to a higher compensatory fluid (and urate) absorption in the proximal tubule (given the distal urinary concentrating defects and chronic state of volume depletion) leading to hyperuricemia. Compared to other ADTKD forms, *UMOD* mutations commonly cause early onset of hyperuricemia and gout which should raise suspicions for ADTKD-*UMOD* [1, 24]. Gout appears much earlier in ADTKD-*UMOD* than in ADTKD-*MUC1* patients (mean ages of 30 vs. 67 years, respectively) [39]. A younger age of onset for gout correlated with an earlier age of onset for ESKD [23]. Age of ESKD onset in ADTKD-*UMOD* ranged between 25 and above 70 years with a median of 54 years. Men usually develop gout more frequently (75%) than women (50%), and median age of ESKD occurs at 50 years of age for men versus 60 years for women [37]. Another study showed that ESKD prior to the age of 30 years is rare and that women develop ESKD later than men [23]. Most ADTKD-*UMOD* patients have missense mutations, and as is often true of other genetic diseases, the type and localization of missense mutation may affect disease progression, particularly if a partially functional gene product is made [37]. The few ADTKD-*UMOD* patients with complete gene deletions typically have a worse clinical outcome [24]. A few cases of ADTKD with homozygous *UMOD* mutations have been identified, and as expected, these patients frequently develop ESKD several decades earlier than the heterozygous family members consistent with a dose-dependent mutant UMOD effect [25, 162, 163]. A dose-dependent effect of *UMOD* mutations has also been shown in different ADTKD-*UMOD* mouse models [164, 165]. The *UMOD* mutation affected the ratio of low versus high molecular UMOD glycoforms which correlated with the age of onset of ESKD and may provide an in vitro scoring system [23]. Alternatively, the position of the *UMOD* mutation may determine the onset of symptoms with patients carrying *UMOD* mutations in the second or third EGF-like domains presenting earlier than those with more distal *UMOD* mutations [37]. A clinical *UMOD*-score has recently been developed to identify the likelihood of ADTKD-*UMOD* [39]. The *UMOD*-score includes positive points for 1) a family history of early gout (<40 years) consistent with autosomal dominant inheritance (+2), 2) CKD of unknown origin (+2), 3) age at gout onset (<30 year +3 points; >30 year +1 point), 4) hyperuricemia (>8.41 mg/dl +3 points, <8.41 mg/dl +1 point), and 5) histological findings compatible with ADTKD (+2 points). Factors that lower the *UMOD*-score include proteinuria/hematuria (−1), diabetes mellitus/uncontrolled hypertension (−1), and enlarged kidneys/cysts (−1). Patients with a *UMOD*-score > 8 are encouraged to undergo a *UMOD* mutation analysis. Those with a score between 5 and 7 are encouraged to have urinary UMOD levels assessed, and if urinary levels are low *UMOD* mutation analysis is recommended. If a facility is unable to test for urinary UMOD levels, assessment of a *UMOD* gene analysis is recommended for patients with a *UMOD*-score > 5 [39].

Pathophysiology

UMOD mutations cause protein misfolding [7, 64, 166]. Proper tertiary protein folding is a requirement for correct sorting in the ER and subsequent processing in the Golgi apparatus [167, 168]. In kidney biopsies of ADTKD-*UMOD* patients, large intracellular deposits were described (Fig. 1c, d), colocalizing with markers of the ER, and loss of UMOD on the apical membrane [6, 7, 63, 64]. Usually, UMOD is targeted to the apical membrane and then released by the serine protease Hepsin into urine where UMOD forms polymers [169] (Fig. 2). Mice lacking Hepsin have significantly reduced Umod secretion with Umod accumulation in kidney cells. Mutant forms of UMOD are not appropriately trafficked to the apical membrane and are also retained in tubular cells [6, 32, 38] (Fig. 3). Patients with ADTKD-*UMOD* have reduced urinary UMOD levels consistent with impaired trafficking and urinary secretion of the mutant protein [6, 32, 38, 64]. Mutant UMOD that is secreted into the urine can also exert a dominant-negative effect on WT UMOD protein, interfering with WT UMOD polymerization [6, 7, 38, 170]. Over 135 *UMOD* mutations, distributed mostly in the N-terminal epidermal growth factor (EGF)-like domains I-IV and the cysteine-rich D8C domain, have been identified [78]. The vast majority of *UMOD* mutations are missense mutations, and <5% of mutations are in-frame deletions. While no frequent or founder *UMOD* mutations has been identified to date, approximately 60% of all UMOD mutations affect 1 of the 48 conserved cysteine residues and, as such, disrupt formation of at least 1 of the 24 intramolecular disulfide bonds, leading to protein misfolding. Cellular and murine *Umod* mutant models have shown impaired trafficking to the plasma membrane, decreased secretion, accumulation and retention of mutant UMOD in the ER, often resulting in apoptosis [7, 42, 64, 166, 170–174]. To date, six different mutant mouse models for ADTKD-*UMOD* have been generated, all of which lead to accumulation of mutant UMOD in the ER, reduced urinary UMOD secretion, TAL damage with urinary concentration defects, and chronic loss of kidney function [42, 164, 165, 175, 176]. Histology in the mutant mouse models recapitulates the findings from kidney biopsy samples from humans with ADTKD-*UMOD* (Fig. 1). Proteomic analysis of mutant *Umod* mice has shown aberrant activation of the UPR, ER stress, and mitochondrial dysfunction [165, 175, 177, 178] (Fig. 3). Thus, ADTKD-*UMOD* may represent an ER storage disease leading to upregulation of UPR and apoptosis in response to accumulation of misfolded protein within the ER

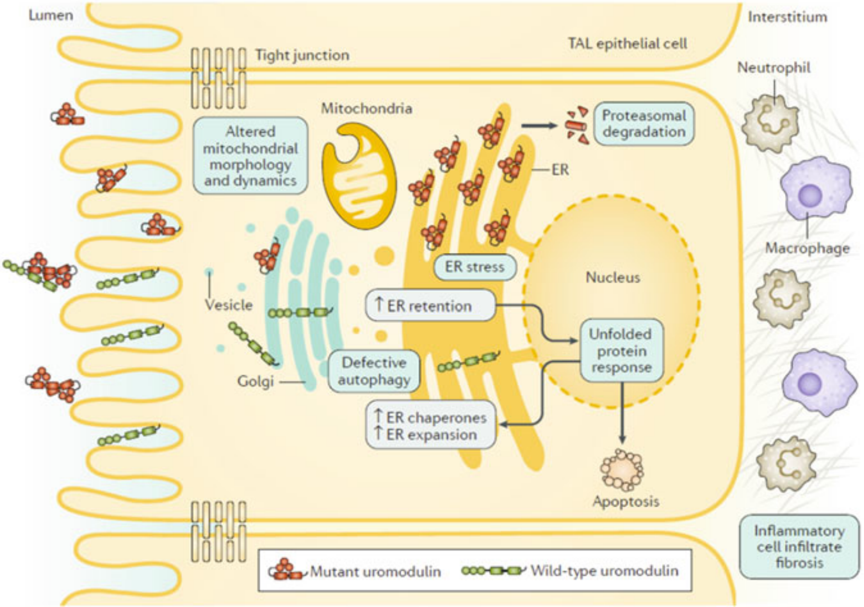
Lumen
Interstitium
Tight junction
TAL epithelial cell
Neutrophil
Mitochondria
Altered mitochondrial morphology and dynamics
Proteasomal degradation
ER
Macrophage
ER stress
Nucleus
Vesicle
↑ ER retention
Unfolded protein response
Defective autophagy
Golgi
↑ ER chaperones
↑ ER expansion
Apoptosis
Inflammatory cell infiltrate fibrosis
Mutant uromodulin
Wild-type uromodulin

Fig. 3 Mutant versus wild type UMOD trafficking and function in renal tubules. Mutant UMOD is retained within the tubular cells of the TAL and the DCT and aberrantly activates different cellular pathways. Accumulation of mutant UMOD in the endoplasmic reticulum (ER) causes ER stress and stimulates the unfolded protein response. Chronic induction of the unfolded protein response causes activation of inflammation and cell death. Downstream consequences of mutant UMOD are defective autophagy and altered mitochondria morphology and dynamics. Mutant UMOD which escapes the ER quality control or proteasomal degradation can undergo trafficking to the apical membrane. Secreted mutant UMOD may interfere with polymerization of wild-type UMOD. This figure was published in [2]

(Fig. 3) [172, 179]. A number of chemical agents, enumerated below, decreased apoptosis in vitro but failed to do so in vivo. In addition to UPR activation, the C147W *Umod* mouse had reduced autophagy and elevated levels of proapoptotic mediators including TNF-α [176]. A different mutant ADTKD-*UMOD* mouse model revealed early onset of inflammation followed by pro-fibrotic signals that sustained disease progression [180]. Interfering with TNF signaling by administering a fusion protein that blocked the TNF receptor (TNFR) ameliorated apoptosis, fibrosis, and kidney failure in that mouse [176]. Mutant UMOD also affects tubular transport of Na^+, K^+, Ca^{2+}, and Mg^{2+} by interfering with NKCC2, ROMK, TRPV5, and TRPM6 trafficking and activity [42, 47, 99, 113, 116]. The different ADTKD-*UMOD* mouse models support the notion that many of the defects associated with *UMOD* mutations lead to mutant forms of UMOD protein that cause a dominant negative effect on the wild-type protein; these findings are supported, because complete *Umod* knockout mice have no abnormal histological kidney findings [181].

Diagnostic Testing

In addition to mutation analysis for *UMOD*, which is provided by many commercial genetic-testing laboratories, assessment of urinary UMOD levels has been a good screening tool to help in the diagnosis of ADTKD-*UMOD*. Spot urine for UMOD is also recommended as a part of a *UMOD*-score [39]. Unfortunately, urinary UMOD analysis may not be available in many countries, in which case *UMOD* mutation analysis has been recommended [39]. Kidney biopsies can be specifically stained for UMOD to demonstrate UMOD storage defects in the TAL (Fig. 1) [63].

Therapeutic Approaches

In cell culture models of ADTKD-*UMOD*, aberrant apoptosis was partially blocked by colchicine, topiroxostat, probenecid, and the chaperone, sodium 4-phenylbutyrate (4-PBA) [170, 172, 179]. The latter improved secretion of mutant UMOD. However, in two different *Umod*-mutant mouse models, the beneficial effects of 4-PBA were not reproduced [182]. Blockade of proapoptotic TNF-α signaling in the C147W *Umod* mouse with the recombinant fusion protein TNFR:Fc prevented decline of renal function in the mutant mice [176]. Administration of topiroxostat, a xanthine oxidase inhibitor, to mutant C112Y UMOD cells, decreased apoptosis rates [179]. Stimulation of autophagy by mTOR inhibitors also increased secretion of mutant C147W UMOD in vitro [176]. A small molecule that targets TMED9, a cargo receptor, was shown to reroute one mutant UMOD protein variant into lysosomes for degradation in tissue culture [57] and as such has therapeutic potential.

ADTKD-MUC1 Is Caused by Mutations in Mucin-1

In contrast to UMOD which has been well known to the Nephrology community, Mucin-1 (MUC1) has surfaced more recently to the nephrology community as the causative gene for ADTKD-MUC1 [10, 183]. The *MUC1* gene (OMIM 158340) is located on chromosome 1q22 and encodes a highly glycosylated epithelial transmembrane protein which is strongly expressed along the TAL, distal nephron, and collecting ducts (Fig. 4a) [184–186]. MUC1 is involved in intracellular signaling, cell-cell, and cell-matrix interactions [187–189] (Fig. 4b). The MUC1 protein contains a N-terminal subunit consisting of signal peptide, a variable nucleotide tandem repeat (VNTR), a sea urchin sperm protein, enterokinase, agrin (SEA) domain, and a C-terminal subunit containing extracellular, transmembrane, and cytoplasmic domains (Fig. 4c) [189]. The hallmark of Mucins is the VNTR domain (Fig. 4c). Each VNTR repeat contains 20 amino acids with the entire VNTR region encoding a repetitive sequence of amino acids which is crucial for the extensive *O*-glycosylation of MUC1 [189]. The length of the VNTR region ranges from 20 to 125 repeats dependent on the inherited paternal and maternal alleles. Similar to UMOD, MUC1 is secreted in the urine [91, 190]. In contrast to UMOD, which is a glycosylphosphatidylinositol-(GPI)-anchored membrane protein, MUC1 is a cell membrane-associated protein, with the N-terminal subunit of MUC1 being tethered to the MUC1 C-fragment in

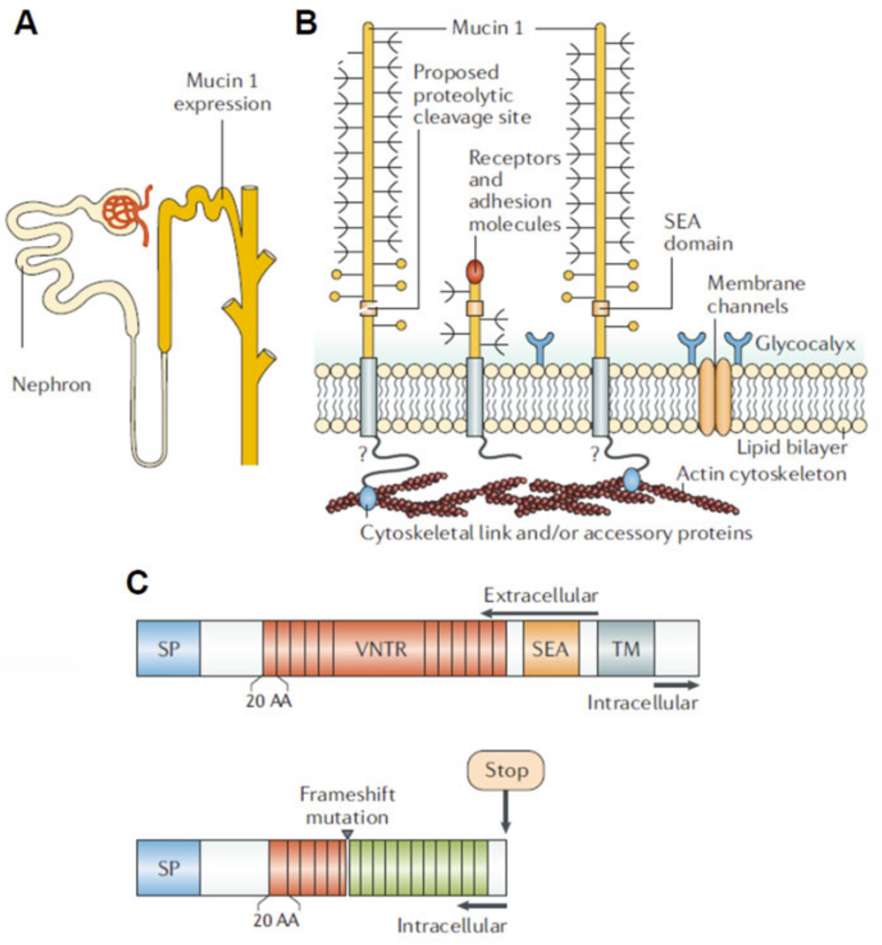
A
Mucin 1 expression
Nephron
B
Mucin 1
Proposed proteolytic cleavage site
Receptors and adhesion molecules
SEA domain
Membrane channels
Glycocalyx
Lipid bilayer
Actin cytoskeleton
?
?
Cytoskeletal link and/or accessory proteins
C
Extracellular
SP
VNTR
SEA
TM
20 AA
Intracellular
Stop
Frameshift mutation
SP
20 AA
Intracellular

Fig. 4 Wild type and mutant MUC1 protein sequence and expression. (**a**) MUC1 is expressed in the TAL, the distal nephron, and the collecting ducts. (**b**) MUC1 is a cell membrane-associated protein, with the N-terminal subunit of MUC1 being tethered to the MUC1 C-fragment in a noncovalent fashion, which can undergo autoproteolysis. MUC1 covers the apical surfaces of epithelial cells in multiple organs. The protein contains an N-terminal and extracellular α subunit which contains a variable number of tandem repeats (VNTR), and each tandem repeat (TR) encodes 20 amino acids (AA). The VNTR is very important for *O*-glycosylation of MUC1. The C-terminal cytoplasmic β subunits anchor MUC1 to the membrane and possibly the cytoskeleton. The extracellular MUC domains protect luminal epithelial surfaces. The MUC1 intracellular domains are involved in signaling via phosphorylation and protein-protein interactions. (**c**) Wild-type MUC1 protein structure includes the signal peptide (SP) (blue) which is required for MUC1 translocation to the ER, the VNTR (red), the sperm protein, enterokinase, and agrin (SEA) domain (orange) and the transmembrane domain (TM) (gray). Auto-proteolytic cleavage in the SEA domain forms the heterodimeric MUC1 protein complex consisting of the N-terminal extracellular α subunit and the C-terminal cytoplasmic β subunits and leaves a transmembrane (TM) domain in the plasma membrane. The mutated MUC1-fs protein contains the SP domain, part of the WT VNTR, and a novel VNTR segment (green) after the frameshift mutation (triangle). MUC1-fs do not include C-terminal sequences required for cellular signaling, post-translational processing, membrane targeting, and MUC1 recycling. (This figure was published in [2])

a noncovalent fashion [191]. While UMOD is cleaved at the C-terminus of the protein and released into the ultrafiltrate, MUC1 undergoes autoproteolysis in the SEA domain, and the N-terminal part of MUC1 is then released into the tubular filtrate [191, 192] (Fig. 4a, b). The identification of mutations within *MUC1* as responsible for a variant of ADTKD was complicated by the location of the *MUC1* mutations within the VNTR region. In most ADTKD-*MUC1* patients, a cytosine duplication occurs within any one of the VNTR repeats within the tract of seven cytosine residues. The insertion of another cytosine residue within the VNTR creates a frameshift and results in a newly encoded frameshift peptide (MUC1-fs). These mutations occur at the C-terminal of the signal sequence, so that the MUC1-fs protein is properly trafficked to the ER, but later accumulates within tubular cells (see below). As the VNTR is a highly GC-rich region and as the insertion occurs in a different unit of the VNTR for each family, the detection of the *MUC1* insertions has been highly challenging [10].

Protein Function

MUC1 is detected in the developing kidney and is upregulated in renal cell carcinoma [193, 194]. Similar to UMOD, MUC1 forms polymers in urine and contributes to a viscous, mucous layer protecting the epithelial surface of renal tubules and in other organs [188, 195, 196]. MUC1 interacts with epidermal growth factor receptor and correlates with the mitogen-activated protein kinase activation in the mammary gland, raising concern for a role of MUC1 in breast cancer [197]. MUC1 appears to also have a causative role in pancreatic and colon cancer and is a target for immunotherapy with a vaccine against the protein [198, 199]. MUC1 promotes metastasis in other types of cancer, and antibody therapy is under development to prevent metastases [200–202]. MUC1 promotes metastasis by interacting with the lectin galectin-3 [203, 204]. Despite strong MUC1 expression in breast, lung, and gastrointestinal tissues, patients with ADTKD-*MUC1* do not have a higher incidence of breast, lung, or gastrointestinal cancer [31]. Similar to UMOD, MUC1 appears to protect against AKI by activating hypoxia-inducible factor 1α and β–catenin signaling [205, 206]. In addition, calcium-containing kidney stone-formers were found to have lower urinary MUC1 levels compared to nonkidney stone formers [186]. Mechanistically, MUC1 stabilizes the abundance of the apical calcium channel TRPV5 in the luminal membrane, thereby reducing the risk for hypercalciuria and calcium-containing crystals in normal individuals [186].

Clinical Presentation

Patients with ADTKD-*MUC1* typically present earlier than those with ADTKD-*UMOD* and have gout and/or hyperuricemia less frequently [39]. ADTKD-*MUC1* patients typically have a bland urine sediment, and no increased risks of UTIs, kidney stones, or polyuria [31]. The presentation is often more subtle than in ADTKD-*UMOD* with slow, progressive CKD as the main features [2]. In the United States, the Broad Institute, in Boston, is the only clinically certified laboratory to directly test for *MUC1* mutations. Other potential diagnostic tools are currently under development, including detection of the MUC1-fs protein by immunofluorescence in urinary cells [60]. Single-molecule real-time sequencing and SnapShot minisequencing also seem to be promising means for eventually diagnosing ADTKD-*MUC1* [49, 58, 59].

Pathophysiology

The chromosomal locus for mutations that cause forms of ADTKT-*MUC1* (formerly known as MCKD type 1) was well-known for years, and many candidate genes were identified [9, 207, 208]. Extensive mutation analysis of candidate genes with Sanger sequencing did not reveal the causative gene mutations, including in *MUC1* [209]. At that time, it was impossible to sequence through long stretches of repetitive DNA through the *MUC1* VNTR (up to 7.5 kB) (Fig. 4c). Many years later by applying cloning, capillary sequencing, de novo assembly, and a probe-extension assay, causative *MUC1* mutations were identified as a cytosine duplication within any one of the VNTRs resulting in the mutant MUC1-fs protein [10, 49]. All VNTRs after the frameshift mutation have a new sequence including one cysteine and six basic amino acid residues [10]. This results in MUC1-fs being very cysteine rich, highly positively charged, and natively unfolded. The frameshift mutation leads to a premature stop codon soon after the altered VNTR [10]. This results in a loss of the MUC1 C-terminus which is important for posttranslational processing, forward trafficking to the apical membrane, and recycling of MUC1. The MUC1 C-terminus is required for MUC1 transport via exosomes to Flotillin-2-

organized lipid rafts on the cell membrane [210]. The C-terminus is also crucial for the extensive MUC1 *N*- and *O*-glycosylation. To date, six different *MUC1* frameshift mutations, mostly nucleotide insertions and duplications within the VNTR, have been described [10, 49, 60], all of which result in expression of a MUC1-fs protein [10, 31, 49, 58, 59, 211–213]. Similar to UMOD, the intracellular accumulation of MUC1-fs is thought to cause the pathological effects of the disease. Proteomic analysis of extracellular vesicles shed by the renal tubular cells into the urine from ADTKD-*MUC1* patients found a reduction of proteins involved in vesicle transport and membrane dynamics of exosomes and lysosomes [214]. Consistent with the notion that accumulation of a dominant negative MUC1-fs protein is responsible for the pathological findings, mice with complete *Muc1* deletion do not develop progressive kidney disease [215]. While the abnormal MUC1-fs protein is detected in many different organs including breast, stomach, and sebaceous glands, the kidney is the only organ to develop pathological findings. While the reason for the kidney-specific sensitivity to MUC1-fs is unknown, some have speculated that MUC1-fs may interact with another tubular protein only present in the kidney [10, 31, 58].

Diagnostic Testing

Direct detection of MUC1 mutations is available at the Broad Institute in Boston (https://www.broadinstitute.org/rare-diseases/muc1-kidney-disease-mkd). SNaPshot minisequencing may soon be used to identify the abnormal insertions in the MUC1 VNTR. Alternatives include single molecule real-time sequencing, and development of specific antibodies that detect mutant MUC1-fs in kidney biopsies and urinary cells [58–60].

Therapeutic Approaches

The small molecule BRD4780 has been identified to improve MUC1-fs secretion by targeting a cargo receptor called TMED9 [57]. This small compound reroutes the MUC1-fs-mutant protein to lysosomes for degradation and may eventually be used clinically in patients with ADTKD-*MUC1*.

ADTKD-HNF1B Is Caused by Mutations in Hepatocyte Nuclear Factor 1β

Hepatocyte nuclear factor 1β (HNF1B) is a DNA-binding transcription factor of 557 amino acids encoded by the *HNF1B* gene (formerly known as *TCF2*) (see Table 1) on chromosome 17q12. HNF1B regulates embryonic development and tissue-specific gene expression in epithelial organs including the kidney, liver, intestine, genital organs, and pancreas [216].

Protein Function

In the kidney, HNF1B is expressed in all tubular epithelial cells and controls the expression of different genes involved in membrane transport, cell differentiation, and metabolism. HNF1B binds DNA and modulates gene expression by inducing conformational changes in chromatin structure via its interaction with coactivators and corepressors [216]. HNF1B is widely expressed in the embryonic kidney and is required at multiple steps of kidney development and signaling, and it is essential in branching morphogenesis, nephrogenesis, nephron patterning, and tubulogenesis. Mice with deletion of both *Hnf1b* alleles do not survive postnatally whereas kidney-specific inactivation of *Hnf1b* causes a variant of polycystic kidney disease [69, 217]. *Hnf1b* null mice have pronounced tubular developmental defects, including an absence of proximal, distal, and loop of Henle segments [218]. As a consequence, *HNF1B*-mutant kidneys are hypoplastic with impaired tubular development [219]. Moreover, preclinical studies have shown that HNF1B downregulates cystic kidney disease genes, including *UMOD*, *PKHD1*, and *PKD2*, which explains cyst formation in mutant animals [216, 217, 219]. In the adult kidney, HNF1B controls the expression of genes required for intrarenal metabolism and solute transport by tubular epithelial cells, providing support to the observed phenotype of distal tubular impairment in adult patients with *HNF1B* mutations [46]. Furthermore, HNF1B has apparent roles in epithelial–mesenchymal transition (EMT), Wnt signaling, renal cell fibrosis (through the *TWIST2*-

dependent transcriptional network), and aberrant TGF-β signaling, all of which link mutations in HNF1B with ADTKD [220].

Clinical Presentation

Heterozygous mutations of *HNF1B* are among the most common single-gene mutations found in developmental kidney disease [21]. This condition is often undiagnosed due to its heterogeneous clinical manifestations (Table 3), incomplete gene penetrance, and frequently arising from de novo mutations with no family history of disease [11, 24, 26–28]. De novo mutations occur in approximately 40% of patients, while the remaining affected patients follow a dominant inheritance pattern [221–223]. In humans, *HNF1B* mutations cause congenital abnormalities of the kidney and urinary tract (CAKUT), including renal agenesis/hypoplasia, multicystic dysplastic kidneys (Fig. 5), horseshoe kidneys, and glomerulocystic kidney disease (Fig. 6) [11, 28, 224]. *HNF1B* mutations can also cause ADTKD, often associated with hyperuricemia, hypomagnesemia, hypokalemia, diabetes mellitus, and Müllerian duct abnormalities [27, 220].

Clinically, disease manifestation varies with age. In adults, *HNF1B* mutations cause MODY type 5, which is an early-onset (commonly before 25 years of age) autosomal dominant, non-autoimmune form of diabetes mellitus; these individuals are neither obese nor insulin-dependent and may present with bilateral polycystic kidney disease without proteinuria [30, 223]. In contrast, children carrying *HNF1B* mutations have kidney disease that dominates the clinical picture including CAKUT, ARPKD, and/or early CKD [27, 28, 75]. Moreover, a 1.5 MB microdeletion on chromosome 17q12 which includes *HNF1B* and 14 other genes can cause a wide range of extrarenal clinical features such as hypomagnesemia, genital abnormalities (that may become more evident with age), autism spectrum disorders, attention-deficit/hyperactivity disorder, and facial dysmorphism [43, 225–228]. These extrarenal manifestations should trigger suspicion for *HNF1B* mutations and unrecognized abnormalities of the kidneys and the urinary tract [45]. It appears that the risk for severe CKD is lower in

Table 3 Kidney and extrarenal manifestations of patients with *HNF1B* gene mutations[a]

Organ	Comments
Kidney Bilateral hyperechogenic kidneys Bilateral cysts Glomerulocystic disease Multicystic dysplastic kidney Single kidney Horseshoe kidney Oligomeganephronia Hydronephrosis and megaureter Chronic kidney disease Hypomagnesemia Hypokalemia Chromophobe renal carcinoma (exceptional)	Most frequent cause of kidney dysplasia of monogenic origin Normal or small-sized kidneys Amniotic fluid preserved in prenatal ultrasound Renal failure in adults, rare in children Electrolyte disorders mainly in adults
Endocrine Early-onset diabetes (MODY-5) New-onset diabetes after transplantation Pancreatic cysts Hypoplastic pancreatic body and tail	<5% of MODY-type diabetes Anticalcineuric drugs downregulate HNF1B
Digestive Pancreatic exocrine dysfunction Elevated liver enzymes (common) Neonatal cholestasis (rare)	Casual finding, most patients asymptomatic
Genital Bicornate uterus Uterus didelphys Double vagina Vaginal hypoplasia and absent uterus Cryptorchidism Hypospadias Epididymal cysts Agenesis of the vas deferens	Mayer-Rokitansky-Küster-Hauser syndrome cases in HNF1B deletions
Others Early-onset gout (hyperuricemia)	Gout precedes chronic kidney disease
Development-psycho Epilepsy Autism spectrum disorder Developmental delay	Associated with 17q12 rearrangement

[a] Modified from [2] and [225]

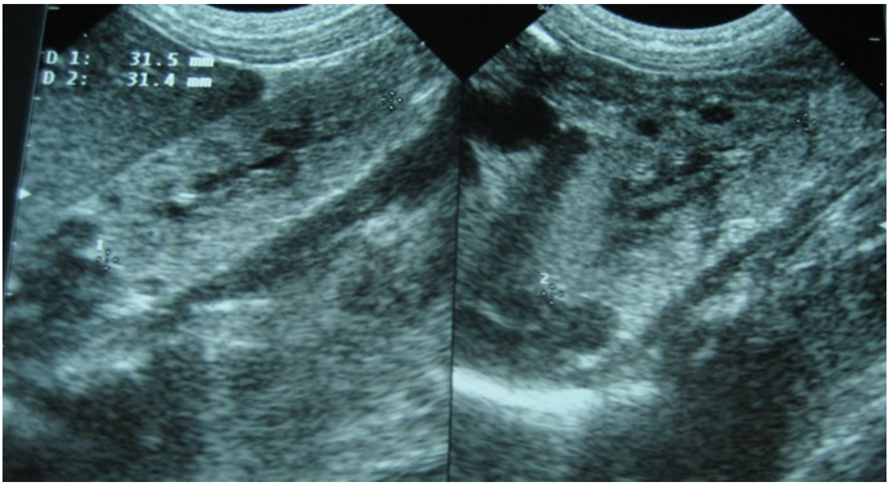

Fig. 5 **Renal ultrasound images from a patient with *HNF1B* mutation**. Renal ultrasound shows bilateral hyperechogenic, cystic, and small kidneys (right kidney length 31.5 mm on the right, and left kidney length 31.4 mm on the left) in a child with a complete *HNF1B* gene deletion

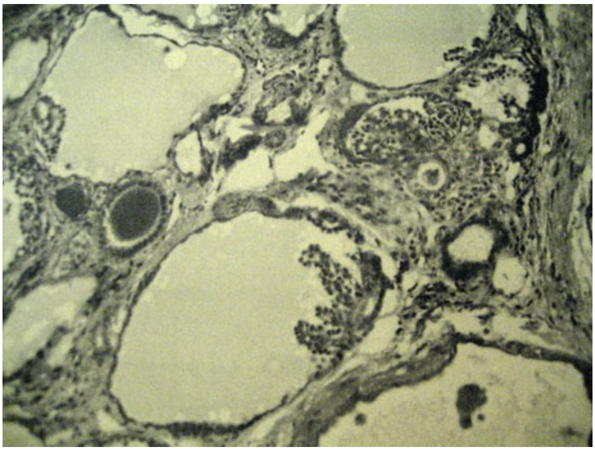

Fig. 6 **Histological image from a patient with *HNF1B* mutation**. Kidney histology of the same patient as in Fig. 5 with a complete *HNF1B* gene deletion shows glomerulocystic disease

patients with *HNF1B* deletions compared to patients with *HNF1B* point mutations, raising the possibility of a dominant negative effect of a mutant protein in the latter [229]. However, both, truncating *HNF1B* mutations and *HNF1B* deletions, can cause tubulointerstitial disease in ADTKD-*HNF1B* [27, 230]. Thus, in humans, *HNF1B* mutations result in an expanded clinical spectrum [2]. Few genotype-phenotype correlations have been identified, in part because the severity of renal disease is highly variable even within families carrying the identical mutation with the exception of affected individuals with 17q12 deletions and developmental disorders [43, 226]. The wide phenotypic spectrum may also indicate that time-specific HNF1B activity is required during organogenesis and the involvement of other modifier genes [69, 231–233]. One-third of the reported human *HNF1B* mutations consist of whole-gene deletions that require specific diagnostic tests to be detected [27].

Pathophysiology

More than 100 pathogenic mutations of *HNF1B* have been reported in humans [27]. The mutations range from intragenic mutations, including missense, frameshift, nonsense, splice site, and small insertions or deletions, to whole-gene deletions and large genomic rearrangements. A lack of HNF1B in renal tubular cells activates the *TWIST2*-dependent transcriptional network which stimulates EMT pathways and abnormal TGF-β signaling. As a result, cells have increased expression of extracellular matrix components that leads to kidney fibrosis [220]. Additionally, HNF1B also regulates a coactivator of the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ). This factor controls mitochondrial biogenesis and function, suggesting that mitochondrial defects may drive pathology in ADTKD [74, 234].

Diagnostic Testing

Mutations in *HNF1B* can be assessed by gene sequencing and testing for gene deletions. Given the high incidence of de novo *HNF1B* deletions, practitioners should test for large deletions with a multiplex ligation-dependent probe amplification (MLPA) assay, as large deletions could be missed by direct gene sequencing. In order to detect the frequently seen 17q12 deletion, a chromosomal microarray or a copy number variation analysis may be required [235].

Therapeutic Approaches

Children with ADTKD-*HNF1B* should be followed by a pediatric nephrologist [1]. Presently, only conservative management is available for ADTKD-*HNF1B*. In case of severe kidney dysplasia resulting in pulmonary hypoplasia, the prognosis can be grave and renal replacement therapy may be needed early. However, in the majority of patients, ADTKD-*HNF1B* is a slow progressive condition [230]. The clinical management should target CKD complications such as metabolic bone disease, anemia, hypertension, hypomagnesemia, hyperuricemia, and short stature as well as extrarenal manifestations such as pancreatic dysfunction and/or correction of genital tract malformations. If a 17q12 microdeletion is suspected or confirmed, psychological evaluation and therapy may be needed for autism and/or ADD [21]. After a kidney transplant, patients with ADTKD-*HNF1B* should be closely monitored for new onset diabetes mellitus after transplant (NODAT) given that tacrolimus-based immunosuppression and steroids aggravate their already higher susceptibility for diabetes [53, 236]. Moreover, tacrolimus can cause or worsen underlying hypomagnesemia in ADTKD-*HNF1B* patients, which is a known key factor for development of NODAT [54, 55, 237, 238]. Practitioners should consider immunosuppressive alternatives such as mTOR inhibitors, belatacept, and/or steroid-sparing regimens [56].

ADTKD-REN Is Caused by Mutations in the Gene Encoding Renin

ADTKD-*REN* is an infrequent cause for ADTKD with only 30 families reported to date [22]. The gene encoding renin (*REN*) is located on chromosome 1q32.1 and encodes preprorenin which is selectively synthesized in the juxtaglomerular apparatus [239]. Preprorenin contains a 23 amino acid N-terminal signal peptide, a 43 amino acid prosegment, and a 340 amino acid mature renin peptide [239]. During renin synthesis, the signal peptide is crucial for trafficking of the nascent preprorenin into the ER lumen (Fig. 7). Inside the ER, preprorenin undergoes further processing to prorenin and glycosylation. Prorenin is then proteolytically processed to the mature endopeptidase renin (Fig. 7) [240]. Prorenin binds its receptor and modifies a variety of signaling pathways [241]. Renin converts angiotensinogen to angiotensin 1, an aspartic protease which thereby stimulates angiotensin and aldosterone action.

Protein Function

As an activator of the Renin-Angiotensin-Aldosterone system (RAAS), renin has different roles in the kidney including renal development, blood pressure regulation, modification of erythropoiesis, and thirst regulation [2]. Stimulators of renin synthesis are hypotension (baroreceptor reflex) and decreased chloride delivery to the macula densa [242, 243]. The significance of renin for renal development was confirmed by patients with compound heterozygous or homozygous mutations in *REN* who develop renal tubular dysgenesis (OMIM 267430) [244]. These patients

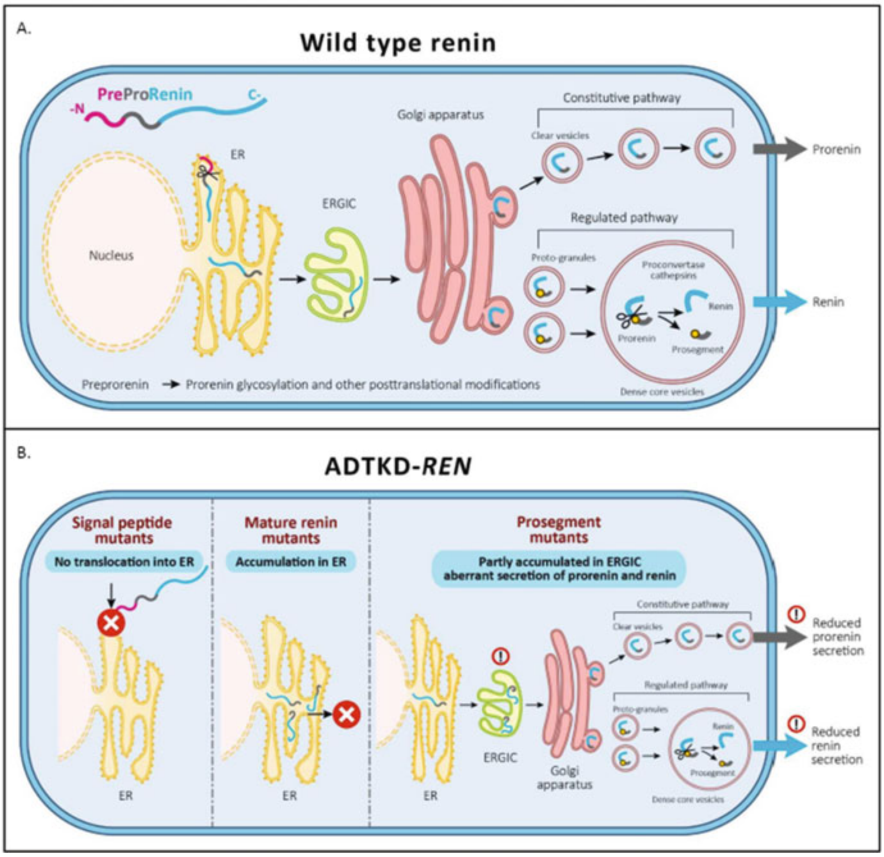
A.
Wild type renin
PreProRenin
-N
C-
ER
Nucleus
ERGIC
Golgi apparatus
Constitutive pathway
Clear vesicles
Prorenin
Regulated pathway
Proto-granules
Proconvertase cathepsins
Renin
Prorenin
Prosegment
Renin
Dense core vesicles
Preprorenin → Prorenin glycosylation and other posttranslational modifications
B.
ADTKD-*REN*
Signal peptide mutants
No translocation into ER
Mature renin mutants
Accumulation in ER
Prosegment mutants
Partly accumulated in ERGIC aberrant secretion of prorenin and renin
Constitutive pathway
Clear vesicles
Reduced prorenin secretion
Regulated pathway
Proto-granules
Renin
Prosegment
Reduced renin secretion
Dense core vesicles
ER
ER
ER
ERGIC
Golgi apparatus

Fig. 7 Wildtype and mutant renin trafficking. (**a**) Wild-type preprorenin translocates in the ER. During translocation, the signal peptide is cleaved, leaving prorenin, which is glycosylated. After the translocation of prorenin through the ER-Golgi Intermediate Compartment (ERGIC), prorenin is cleaved to renin which can be secreted. (**b**) Mutations in the signal peptide interfere with the translocation of the peptide across the ER membrane, leading to abnormal preprorenin location in the cytoplasm. When mutations are located in mature renin, the altered protein is retained in the ER causing ER stress. Mutations in the prosegment result in conformational changes which interfere with protein synthesis, protein folding, and trafficking leading to impaired protein secretion, renin deficiency, and cellular toxicity. This figure was published in [22]

frequently display fetal or perinatal lethality due to renal failure and pulmonary hypoplasia [245]. This condition resembles the fetopathy caused by maternal use of angiotensin II receptor blockers or angiotensin converting enzymes inhibitors (ACEi) during pregnancy [246, 247].

RAAS likely influences erythropoiesis mostly through angiotensin II (Ang II). Ang II type 1 receptors have been identified on the surface of erythroid progenitor cells [248]. Treatment of cultured erythroid progenitor cells with Ang II increased blood forming units, an effect that was prevented by cotreatment with losartan [248]. Ang II is also considered a secretagogue for erythropoietin. Patients with renin-mediated hypertension have higher erythropoietin levels compared to patients with nonrenin-mediated hypertension [249, 250]. One means by which the RAAS affects erythropoietin appears to be from Ang II-mediated vasoconstriction of renal vessels and reduction of postglomerular peritubular blood flow which subsequently decreases oxygen delivery to the renal tubulointerstitium [251, 252]. Ang II also directly interacts with hypoxia inducible factor 1 (HIF-1) which is a key regulator in erythropoiesis [253]. Prepubertal ADTKD-*REN* patients respond well to erythropoietin treatment, although these treatments are usually unnecessary after puberty due to enhanced production of sexual steroids [12, 254] .

Clinical Presentation

ADTKD-*REN* typically presents with early onset of anemia (often in the first year of life), hyperuricemia, and gradually worsening CKD [12, 22, 255–257]. Most patients present before the age of 10 years. The kidney disease progresses with histology that includes tubular atrophy, glomerular scarring, and interstitial fibrosis. Staining for prorenin and renin shows less signal in the granular cells of the juxta-glomerular apparatus and no staining in tubular epithelia [12, 255]. The symptoms and signs of disease depend on the location of the *REN* mutation. Signal peptide mutations are the most common (62% in cohort of 111 affected individuals) and present mostly between childhood and adolescence (mean age at presentation of 19.7 years) with anemia (31% of patients), gout (25%), CKD (22%), and/or AKI (10%) [22]. Prosegment mutations were found in 24% of the aforementioned cohort with most patients presenting before the age of 10 years (61% of this group) with anemia (in 50%) and gout (in 36%). Mutations in the mature protein were the least common with 14% in the aforementioned cohort and were associated with the latest mean age of presentation (37 years) with gout (in 75%) and CKD (in 25%) [22]. Age of onset of ESKD varied between the three different mutation sites from 53 years (signal peptide), to 50.8 (prosegment) to 63.6 years (mature protein). Anemia was described in 91% of ADTKD-*REN* patients in childhood and is associated with a low serum erythropoietin [12, 22]. The anemia in patients with ADTKD-*REN* is reminiscent of anemia seen in patients treated chronically with ACEi or ARBs as the RAAS stimulates erythropoietin synthesis [258, 259]. The anemia in ADTKD-*REN* often resolves during puberty, most likely due to the higher synthesis of sexual hormones and their ability to stimulate erythropoietin production [12]. Other typical features of ADTKD-*REN* include hyperkalemia and mild hypotension which are both caused by the impaired activation of the RAAS. The severity of hyperkalemia usually does not correlate to the degree of renal function decline in patients [12]. Some patients are polyuric and many have gout and hyperuricemia [2].

Pathophysiology

Heterozygous mutations in *REN* lead to apoptosis in renin-producing cells due to intracellular accumulation of abnormal renin protein [12, 22]. While homozygous *REN* mutations are highly lethal due to abnormal kidney development resulting in renal tubular dysgenesis, heterozygous *REN* mutations appear to not overtly perturb kidney development. Heterozygous *REN* mutations of the signal peptide result in impaired ER translocation and processing of Preprorenin, which results in a lower secretion of prorenin and renin. Similar to ADTKD-*UMOD*, cell lines that express a signal peptide mutation of REN had increased ER stress [12].

Diagnostic Testing

One should consider and test for a *REN* mutation if a prepubertal patient has anemia and/or hyperkalemia which is worse than expected based on the degree of CKD [12, 255–257]. Erythropoietin, renin, and aldosterone levels may be reduced, and reticulocyte count may be low. Hyperuricemia is commonly found in ADTKD-*REN* [12]. Immunohistochemical staining of kidney tissue may show reduced staining for renin in the juxtaglomerular apparatus in patients with ADTKD-*REN* [12, 255].

Therapeutic Approaches

Patients may be more susceptible to hypotension and acute kidney injury due to volume contraction. Therefore, patients should be counseled to avoid nonsteroidal anti-inflammatory drugs. Given the tendency for hypotension, the use of ACEi or ARBs may be impractical. If using the aforementioned drugs, patients and families should be instructed to not give them if the patients become dehydrated. Fludrocortisone may be used to improve hypotension and hyperkalemia in these patients [255]. Younger patients may require erythropoietin therapy. CKD slowly worsens over time, and renal replacement therapy may be required, often between 30 and 80 years of age [22].

ADTKD-SEC61A Is Caused by Mutations in SEC61A

After excluding mutations for all known ADTKD-causing genes in two affected family pedigrees, Bolar et al. performed additional genetic analysis including linkage studies and whole-exome sequencing; ultimately, the group identified heterozygous *SEC61A1* mutations as a novel and rare cause of ADTKD [13]. The *SEC61A1* gene is located on chromosome 3q21.3 [13]. In addition to gout, ADTKD-*SEC61A* patients have extrarenal symptoms such as anemia, neutropenia with multiple skin infections, intrauterine growth retardation, cleft palate, and polydactyly.

Protein Function

The *SEC61A1* gene encodes the protein transport protein SEC61 subunit alpha isoform 1 (SEC61α) which assembles with SEC61 subunit beta (SEC61β) and SEC61 subunit gamma (SEC61γ) to form the heterotrimeric protein-conducting channel SEC61 which is a part of the mammalian ER translocon [13]. The SEC61 complex is required to transport newly synthesized secretory proteins into the ER, integrate nascent proteins into membranes, and maintain ER ion permeability barrier. Thus, the human SEC61 complex has a role in translocation of proteins from the cytosol into the ER, and in retrograde transport of multidomain integral membrane proteins from the ER to the cytosol for proteasomal degradation.

Clinical Presentation

Bolar et al. described two unrelated families with ADTKD and congenital anemia (a three-generation family in Belgium, with 7 affected members out of 18 studied, and a family in the United States, with 2 affected individuals out of a pedigree of 8) [13]. In the first family, the clinical phenotype was characterized by autosomal dominant progressive CKD associated with congenital anemia and intrauterine and postnatal growth retardation. Other variable findings included cleft palate, bifid uvula, velopharyngeal insufficiency, preaxial polydactyly, and mild cognitive impairment. Affected individuals exhibited normal blood pressure, normochromic, normocytic anemia responsive to erythropoietin, elevated serum creatinine levels, and mild hyperuricemia, without proteinuria [13]. Renin and aldosterone concentrations were in the lower range. Fractional excretion of sodium and uric acid was low. Ultrasound images demonstrated small, dysplastic kidneys without cysts. Kidney biopsies revealed multiple small foci of tubulo-interstitial lesions and glomerulocystic changes. In the second family, the major clinical findings were congenital anemia and neutropenia, gout in the second decade of life, repeated cutaneous abscesses, and CKD. In the second family, affected members had normal linear growth and intelligence. Renal ultrasound images demonstrated normal-sized kidneys with multiple bilateral simple cysts [13]. In addition to causing kidney disease, *SEC61A1*-pathogenic variants have been shown to cause monogenic plasma cell deficiency in patients [13, 260, 261].

Pathophysiology

SEC61A1 mutations interfere with the function of the translocon pore by affecting the integrity of the plug domain/constriction ring of the translocon pore. These conformational changes result in the accumulation of mutated SEC61α protein in the ER. Moreover, the kidney biopsy of an ADTKD-*SEC61A1* patient showed mislocalized mutant SEC61α protein in the Golgi apparatus and the ER-Golgi intermediate compartment (ERGIC) (similar to ADTKD-*REN*) (Fig. 7) [13]. The detection of mutant SEC61α in the Golgi and ERGIC suggests that the mutant protein is identified by the cell as abnormal and eventually degraded in the ER. It is thought that destabilizing the translocon pore may lead to pathological post-translational modifications, conformational changes, and forward trafficking of secretory and transmembrane proteins that include UMOD, MUC1, and REN. Additional studies in zebrafish embryos confirmed that *sec61al2* variants induced convolution defects in pronephric tubules [13]. Together, these data are consistent with a crucial role of *SEC61A1* for proper tubular organization [13]. Other effects of *SEC61α* mutations include abnormal cellular Ca^{2+} homeostasis and energy metabolism [262]. The impaired functions of the translocon are speculated to either cause or impair the cell to compensate appropriately for ER stress, leading to apoptosis and tissue damage. As noted, chronic ER stress is a major pathogenic mechanism driving development and progression of ADTKD caused by *UMOD*, *MUC1*, and *REN* mutations [7, 10, 12, 171]. Patients with ADTKD-*SEC61α* may have a common pathogenetic mechanism leading to similar clinical symptoms as those with the other types of ADTKD [2].

Diagnostic Testing

Due to the limited number of patients, no specific tests for ADTKD-*SEC61A1* exists, except direct sequencing for *SEC61A1* mutations. Gout and extrarenal symptoms are often present, as noted above. Slowly progressive CKD is a typical feature. Complete blood counts may show anemia and neutropenia. Kidney biopsies may show glomerulocystic changes in addition to tubulointerstitial fibrosis. Urinary UMOD may also be reduced in ADTKD-*SEC61A1* patients.

Therapeutic Approaches

The anemia associated with ADTKD-*SEC61A1* responds well to erythropoietin. Fludrocortisone may be effective for patients with hyperkalemia due to low renin and aldosterone concentrations. Other manifestations of CKD should be treated as per common guidelines.

Treatment of ADTKD

Presently, no specific treatments or recommendations exists for most patients with ADTKD, in part from a lack of published trials. Patients with mild and moderate CKD due to ADTKD should undergo annual follow-up clinic visits. In asymptomatic children of ADTKD patients, laboratory studies may be performed dependent on family preference. Most often, CKD in children affected by ADTKD-*UMOD* or ADTKD-*MUC1* is mild and may not require significant therapy. In contrast, children with *HNF1B* and *REN* mutations may require more intensive early interventions. There are no data on whether angiotensin-converting enzyme inhibitors or angiotensin blockers improve outcomes in ADTKD patients. Given these medications are widely used in pediatric CKD cohorts and generally tolerated well, some advocate attempting treatment with these medications [263, 264]. If these medications are used, losartan may be a good choice in hyperuricemic patients as it lowers uric acid levels due to increased urinary urate excretion [265, 266]. In patients with gout or hyperuricemia, allopurinol or febuxostat may help to prevent future episodes of gout [24, 37]. It remains unclear if allopurinol reduces CKD progression in ADTKD. In a cohort of adult patients with CKD stages 3 and 4, allopurinol did not attenuate in the decline of GFR [267]. Additionally, allopurinol may exacerbate gout and rarely causes severe allergic reactions. As such, allopurinol should be immediately discontinued in cases of rash, which is suspicious for allergy. Allopurinol should also be avoided in pregnancy as it can cause cleft palate and other facial anomalies in the fetus. Diuretics should be used carefully as they may worsen volume depletion, hyperuricemia, and gout [268]. Generous water intake may be helpful to compensate for urinary concentration defects. Low salt intake is typically not recommended in children with CKD as that may aggravate hyperuricemia and volume depletion in ADTKD-*UMOD* and ADTKD-*REN*. As in all forms of CKD, nonsteroidal anti-inflammatory drugs should be avoided in ADTKD patients, especially ADTKD-*REN* patients that appear to be very susceptible to acute kidney injury from these medications [1]. As in other forms of CKD, management should include control of blood pressure, lifestyle modifications, and treatment of hyperuricemia, anemia, metabolic acidosis, bone disease, electrolyte abnormalities, and cardiovascular disease. Anemia due to ADTKD-*REN* and in all other more advanced stages of CKD from other ADTKD forms is amenable to erythropoietin therapy. In patients with ADTKD-*REN*, fludrocortisone may improve hypotension and hyperkalemia. That said, practitioners need to balance the potentially beneficial effects of fludrocortisone against possible risks of mineralocorticoid-induced aggravation of interstitial fibrosis. As such, fludrocortisone should be avoided in patients with severely declining kidney function, hypertension, or edema. Possible future treatment options for ADTKD may include small biological molecules. For example, ADTKD-*MUC1* human-iPCS-derived kidney organoids and kidneys from heterozygous MUC1-fs knockin-mice had markedly reduced MUC1-fs accumulation when treated with the small molecule BRD4780 [57]. Presently, dialysis and/or kidney transplantation still remain the only treatment for ESKD due to ADTKD. To date, no cases of recurrent ADTKD has been reported in the allografts [269]. ADTKD-*HNF1B* patients with diabetes mellitus may require combined kidney/ pancreas transplantation.

Conclusion

ADTKD is a relatively newly characterized group of tubulointerstitial nephropathies which frequently result in the accumulation of a misfolded, mutant protein in the renal tubular cells. The causative mutated genes encode the aberrant protein that accumulates (e.g., UMOD, MUC1, or REN), encode proteins required for other protein processing in the ER (e.g., SEC61A1), or encode factors required for proper expression of other target genes (e.g., *HNF1B* regulation of UMOD expression). Often, accumulation of aberrant proteins with ADTKT leads to ER stress and ADTKD. Similarly, *DNAJB11* mutations result in an ADPKD-ADTKD phenotype that appears secondary to ER stress, as DNAJB11 is a key chaperone that participates in ER quality control [71]. Most of the ADTKD forms can present in childhood but typically do not lead to ESKD until adulthood. As clinicians and researchers have become more aware of ADTKD, it is now recognized as a major cause of monogenic kidney disease [1]. A challenge in making the correct diagnosis is the lack of specific symptoms. In addition, ADTKD poses an ethical dilemma as to whether the practitioner should assess for causative mutations as there are no specific therapies at present. Quality of life is rarely affected in children with ADTKD unless they have gout, diabetes, hypomagnesemia, neuropsychological symptoms, or anemia. Better biomarkers may help in making the correct diagnosis in the future. International registries and ongoing preclinical studies will hopefully help to identify better therapeutic options. Promising therapies include the small molecule BRD4780, which may become a targeted therapy for ADTKD-*MUC1*. Other future treatments could include the use of antisense oligonucleotides to reduce the production of mutant proteins (as happens with *UMOD*, *MUC1*, and *REN* mutations), chemical chaperones to aid with secretion of mutant proteins, inhibition of inflammatory TNF signaling, and/or proteostasis modulators [2]. As targeted therapies become available, pediatric nephrologists may be critical in initiating early treatment to remove mutant proteins from renal tubular cells before permanent damage has happened [270].

Cross-References

- ▶ Autosomal Dominant Polycystic Kidney Disease
- ▶ Autosomal Recessive Polycystic Kidney Disease
- ▶ Chronic Hemodialysis in Children
- ▶ Epidemiology and Management of Chronic Kidney Disease in Children
- ▶ Nephronophthisis and Related Ciliopathies
- ▶ Pediatric Tubulointerstitial Nephritis
- ▶ Peritoneal Dialysis
- ▶ Progression of Chronic Kidney Disease and Nephroprotection in Children

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Part VIII

Urinary Tract Disorders

Urinary Stone Disease and Nephrocalcinosis

54

Vidar O. Edvardsson and David J. Sas

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Abstract

A significant increase in the frequency of childhood urinary stone disease has been observed during the last two decades. A recent study observed an increase in the incidence of symptomatic kidney stones from 7.9 per 100,000 children in 1996 to 18.5 per 100,000 in 2007. The highest incidence was observed in females aged 14–17 years. These findings have been replicated by others. The reason for this increase is not clear though many hypotheses have been proposed. Most affected children have idiopathic calcium stone disease while monogenic disorders, sometimes associated with chronic kidney disease, likely account for approximately 10% of pediatric kidney stone cases. Both environmental and hereditary factors significantly contribute to the risk of stone formation. All children with urinary stone diseases deserve a meticulous risk factor evaluation which forms the basis for individualized and targeted treatment of the underlying disorders.

Keywords

Urinary stone disease · Nephrolithiasis · Urolithiasis · Kidney stones · Nephrocalcinosis

Synonyms

Urinary stone disease; Nephrolithiasis; Urolithiasis; Kidney stones; Nephrocalcinosis

Epidemiology of Childhood Urinary Stone Disease

Urinary stone disease is a common health problem in all parts of the world with an estimated lifetime prevalence of approximately 10–12% in men and 5–6% in women [1, 2]. Although the epidemiology of childhood urinary stone disease is less well defined than in adults, three recent population-based studies [3–5] have suggested a significant increase in the frequency of pediatric urolithiasis over the past few decades. A study conducted in the state of South Carolina [3] found an overall increase in the incidence of symptomatic kidney stones in children <18 years of age, from 7.9 per 100,000 children in 1996 to 18.5 per 100,000 in 2007. In that study the highest incidence increase was in females aged 14–18 years, followed by girls aged 9–13 years. Overall, the risk of developing stone events was 10 times greater in children aged 14–18 years than for those 0–3 years of age [3]. A significant increase in the incidence of childhood urinary stones was also observed in a recent population-based study from Iceland [5]. The total annual incidence increased from 3.7/100,000 in the years 1985–1989 to 11.0/100,000 in the years 1995–2004 and then decreased to 8.7/100,000 in 2010–2013. The incidence rise was highest in girls aged 13–17 years, in whom it increased from 9.8/100,000 in 1985–1989 to 39.2/100,000 in 2010–2013. Findings were similar in the third study [4] that was based on the Rochester Epidemiology Project [4]. In all three studies, the incidence over time did not change significantly in prepubertal children [3–5].

Published data on childhood kidney stone recurrence are limited. A retrospective population-based study of 190 Icelandic individuals who had their first stone at the age of <18 years, showed a recurrence rate of 26%, 35%, and 46% at 5, 10, and 20 years, respectively [6]. Stone recurrence was not affected by sex or age at first stone diagnosis. A retrospective single-center investigation on US children aged 3–18 years documented stone recurrence in approximately 50% of cases at 3 years [7]. Based on these data, the rate of stone recurrence appears to be similar in children and adults.

Multiple studies of urinary stone disease in both children [5, 8, 9] and adults [1, 10] have shown male preponderance. Recent pediatric studies have, however, found kidney stone disease to be more common in girls [3, 5, 11, 12], particularly in the age group of 10–17 years [3, 5, 13]. Conversely, recent studies have suggested a decrease in male predominance in young adults [10, 14, 15]. Taken together, these data suggest that the recently reported female predominance in childhood stone disease may be antedating a shift in the sex ratio in adults.

The reasons for the increased incidence of pediatric urinary stone disease are not clear and may include increased intake of sodium and fructose, decrease in the intake of calcium and water, and increased use of antibiotics [16]. While obesity has been shown to be a risk factor for adults [17, 18], epidemiological data in children and adolescents do not suggest a link between higher body mass index (BMI) and stone formation [16, 19]; some studies have even observed a significant association between low body weight and kidney stone formation in US children [16].

Since the higher risk of stone formation in obese adults is, at least in part, mediated by lower urine pH, higher urine pH observed in children could protect against obesity-induced urolithiasis in young subjects [20].

Several studies have shown an inverse relationship between dietary calcium intake and the risk of symptomatic kidney stones in adults [21–23]. A low calcium diet in adults increases urinary oxalate excretion, while a diet containing normal amounts of calcium and reduced amounts of animal proteins and sodium decreases urinary oxalate excretion and the risk of developing symptomatic kidney stones [24]. Dietary calcium is thought to decrease intestinal oxalate absorption by binding free oxalate [25].

Low fluid intake is a clear risk factor for nephrolithiasis [23]; US children drink on average less than before, which may contribute to the observed rise in the incidence of urolithiasis in this population [26]. Low fluid intake and increased environmental heat increase urinary supersaturation and the risk of urinary stone formation [27, 28]. Conversely, increased fluid intake reduces stone recurrence [29–31].

Finally, stone formation may be associated with antibiotic use through disruption of the normal microbiome [32]. For example, *Oxalobacter formigenes* is an intestinal bacteria that that relies on oxalate degradation to meet most of its energy and carbon needs [33]. Reduced *Oxalobacter* gut colonization due to antibiotic use may increase the bioavailability of oxalate for intestinal absorption, and consequently urinary oxalate excretion. Antibiotic use may not, however, account for the increasing incidence of urinary stone disease in children in the U.S. as recent epidemiologic data show decreasing use of antibiotics in American children [34, 35].

The prevalence of kidney stones varies between ethnic groups and geographical areas [36]. The prevalence of kidney stones in adults has been reported to be 1–5% in Asia, 5–9% in Europe, 13% in North America, and 20% in Saudi Arabia [37]. In Western industrialized nations, kidney stones are most common in non-Hispanic Caucasians, while the lowest risk is observed in African Americans [1, 36]. Similar differences between ethnic groups have also been observed in pediatric studies [3, 12, 38]. A considerable regional variability exists in stone prevalence in North America with the highest reported probability of stone formation in the South and the lowest in the West [1]. However, a recent retrospective study of nearly 4,000 hospital admissions for urinary stone disease in pediatric patients found stone hospitalizations to be more frequent in the North and Central US, compared to the Southern states [12]. Similar to adult data, stone events during summer months are associated with an increased risk of hospitalization in children [12].

Children and adolescents in Western countries predominantly form calcium stones. In a recent large US study, 89% of the stones contained calcium salts [39]. The study, which is the largest one reported to date, included 5,245 first kidney stones formed by children aged 1–17 years and analyzed between 2000 and 2009. Calcium oxalate and magnesium ammonium phosphate (struvite) were found significantly more often in females than in males (86% vs. 79% and 4.8% vs. 3.8%, respectively); uric acid stones were more commonly seen in males than in females (1.4% vs. 0.6%). The percentage of calcium oxalate stones increased with age, while stones containing calcium phosphate were more common in young children, possibly because of higher urine pH in this age group. Struvite and ammonium acid urate containing stones decreased with age. Calcium was found in 89% of the stones, calcium oxalate in 83%, calcium phosphate in 61%, and struvite in 4.4%. Other less frequently observed components were ammonium acid urate (2.8%), cystine (2.3%), uric acid (0.9%), and calcium carbonate (0.4%). The relative prevalence of the different stone types may also be changing. A single center study analyzing urinary stone composition in 179 children found a significantly higher proportion of calcium phosphate stones (60% vs. 47%) in the years 2001–2010, compared to the years 1992–2000 [40].

Finally, stone composition in developing countries including India, Pakistan, and in Southeast Asia and the Middle East, where urolithiasis is endemic, is different from that in the Western world [41, 42]. Calculi are more commonly observed in the bladder and are predominantly composed of ammonium acid urate, which is associated with the excretion of high urinary acid load caused by rice- or cereal-based diet and frequent episodes of dehydration, combined with low dietary phosphate, which reduces urinary phosphate excretion [43]. The association between stone types and various clinical conditions is illustrated in Table 1.

Nephrocalcinosis

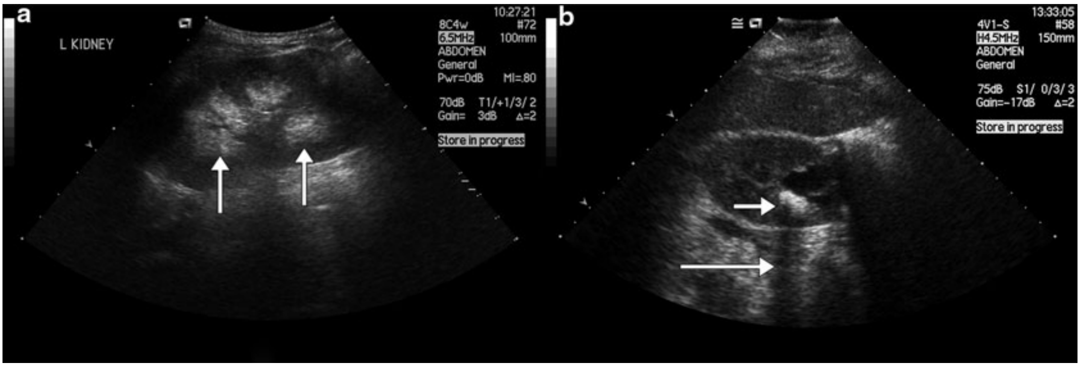
The term “nephrocalcinosis” (Fig. 1) indicates a diffuse deposition of calcium oxalate or calcium phosphate in renal tubules and/or in the renal interstitium [45]. Compared to urinary stone disease, the pathophysiology of nephrocalcinosis is less well defined, and involves, at least in part, increased urinary excretion of calcium, oxalate, phosphate, and/or reduced excretion of citrate [45]. Nephrocalcinosis can cause renal parenchymal inflammation and fibrosis, which may lead to chronic kidney disease (CKD) [46]. It is a prominent feature of conditions that cause hypercalciuria, such as primary hyperparathyroidism, genetic tubular diseases, and chronic use of loop diuretic use (especially in infants) and conditions causing hyperoxaluria, including excessive vitamin C intake or ethylene glycol ingestion [47–50].

While most urinary stones form in otherwise healthy people, nephrocalcinosis is usually caused by an underlying disorder [51] that requires a thorough evaluation, including metabolic and genetic studies. The severity of nephrocalcinosis is difficult to evaluate. Grading systems, based on ultrasound imaging, have been proposed, but are not routinely used in the clinical setting [52].

Increased risk of nephrocalcinosis has been observed in preterm infants [44, 51]. Earlier studies reported renal calcifications in 16–64% of very low and extremely low birth weight infants or premature babies of less than 32 weeks’ gestation but more recent studies suggest lower prevalence rates (7–41%) [48]. The risk of renal calcifications in small infants is associated with the use of drugs (in particular furosemide), vitamins, electrolyte supplementation, metabolic acidosis, longer duration of mechanical ventilation, and parenteral nutrition [44, 48, 51]. Furosemide, steroids, methylxanthines, vitamin D, and supplementation with

Table 1 Kidney stone type and associated conditions [44]. (Modified with permission from Dawn Milliner, MD, Mayo Clinic, Rochester Minnesota and Springer Science and Business Media)

Stone type	Associated conditions
Calcium oxalate	Idiopathic stone disease
	Hypercalciuria, idiopathic, or of any cause
	Dietary hyperoxaluria
	Enteric hyperoxaluria
	Primary hyperoxaluria
	ADPKD
	Cystic fibrosis
	Ketogenic diet (mixed calcium stone)
	Drug-induced metabolic stones
Calcium phosphate	Idiopathic stone disease
	Hypercalciuria, idiopathic, or of any cause
	RTA
	Dent disease type I
	Dent disease type II (*OCRL* mutations)
	Drug-induced metabolic stones
	Ketogenic diet
Magnesium ammonium phosphate (struvite)	Infection related stones
Carbonate apatite	
Uric acid	Hyperuricosuria
	Metabolic syndrome
	Ketogenic diet (pure and/or mixed with calcium)
	HPRT deficiency, partial or complete
	Hereditary renal hypouricemia
	Tumor lysis syndrome
	Glycogen storage disease, type 1
	ADPKD
	Wilson's disease
2,8-Dihydroxyadenine	APRT deficiency
Cystine	Cystinuria


a
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8C4w #72
6.5MHz 100mm
ABDOMEN
General
Pwr=0dB MI=.80
70dB T1/+1/3/ 2
Gain= 3dB Δ=2
Store in progress
b
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4V1-S #58
H4.5MHz 150mm
ABDOMEN
General
75dB S1/ 0/3/ 3
Gain=-17dB Δ=2
Store in progress

Fig. 1 Renal ultrasound examination. (**a**) Nephrocalcinosis in a 6-year-old boy with distal renal tubular acidosis. (**b**) An 8 mm shadowing (short arrow) stone (long arrow) in the right kidney of a 12-year-old boy with recurrent stone disease

calcium and sodium increase renal calcium excretion and may cause hypocitraturia in very low birth weight infants [51]. Risk factors for hyperoxaluria in premature children include parenteral nutrition and enteral feeding with formula, as opposed to breast milk [48]. A positive family history of urinary stone disease, male gender, and white race also increase the risk of nephrocalcinosis in preterm infants [51]. Kidney stones are not observed more frequently in former preterm infants, even in those with a history of severe nephrocalcinosis during the neonatal period [48, 53]. Spontaneous resolution of nephrocalcinosis may occur during the first years of life [48]. Long-term follow-up ultrasound studies of preterm neonates have shown the persistence of nephrocalcinosis in 34%, 15%, and 10% of children after 1.3, 2.5, and 7.4 ± 1.0 years, respectively [48, 53].

Transient renal medullary hyperechogenicity in neonates, mimicking nephrocalcinosis, is a benign condition caused by Tamm Horsfall/Uromodulin tubular precipitations which typically resolves within a few weeks [54].

Risk Factors for Urinary Stone Disease

Urine metabolic risk factors for calcium kidney stones in the absence of systemic diseases include idiopathic hypercalciuria, hyperoxaluria, hypocitraturia, low urine volume, and hyperuricosuria [19, 55, 56]. These factors have been reported in 40–95% of first time pediatric stone formers [11, 57, 58]. Table 2 shows normal values for urinary solute excretion in children <18 years of age, and metabolic abnormalities associated with urolithiasis are listed in Table 3.

Idiopathic Hypercalciuria

Idiopathic hypercalciuria is defined as the renal overexcretion of calcium, despite normal serum calcium levels, and in the absence of an underlying cause (Table 2) [63]. It is the most common risk factor for urinary stone disease, which is observed in 50–80% of pediatric kidney stone patients [11, 64]. A recent carefully designed study by Bergsland et al. [19] found that hypercalciuria is the principal risk factor for calcium stone formation in children; in the same study the frequency of other risk factors for stone formation, such as hyperoxaluria, hypocitraturia, abnormal urine pH, or low urine volume, were similar between stone formers and controls. The mechanisms of idiopathic hypercalciuria include [1] primary (not vitamin D-mediated) increase in intestinal calcium absorption (type 1 absorptive hypercalciuria) or excess of 1,25-$(OH)_2D3$-mediated calcium absorption (type II absorptive hypercalciuria); [2] decreased renal calcium reabsorption (renal hypercalciuria) or decreased renal phosphorus reabsorption (type III absorptive hypercalciuria), and/or [3] enhanced bone resorption (resorptive hypercalciuria) [65]. Bone mineral density is reduced in individuals with idiopathic hypercalciuria; a large cross-sectional study from the Third National Health and Nutrition Examination Survey (NHANES III) showed that male subjects with history of kidney stones had lower femoral neck bone mineral density and higher fracture risk than control subjects [66]. An inverse linear correlation between age and bone mineral content has been observed in children with idiopathic hypercalciuria suggesting that osteoporosis may start in childhood [67].

Dietary factors affecting urine calcium excretion include the intake of animal protein, sodium, potassium, phosphorous, and calcium [65]. A direct association has been established between animal protein ingestion and the risk of kidney stone formation in adults in large prospective cohort studies [21, 23] and clinical trials [68, 69]. Animal protein ingestion produces an acid load, which may promote skeletal calcium losses and hypercalciuria [70–72]. High sodium intake increases urinary calcium excretion [73] by inhibiting tubular calcium reabsorption [65]. Conversely, low sodium intake decreases urinary calcium excretion [74]. Dietary potassium has an opposite effect on urinary calcium excretion; the primary sources of potassium are the organic anion salts of fruits and vegetables, which increase the oral alkali intake, and reduce the net acid excretion [65]. Low dietary phosphorous and increased renal

Table 2 Reference intervals for urinary solutes [59–62]

<table>
<tr><th>Urinary Solute</th><th>Age</th><th colspan="4">Random</th><th colspan="2">24-h</th></tr>
<tr><th></th><th></th><th colspan="2">SI Units</th><th colspan="2">Conventional Units</th><th>SI Units</th><th>Conventional Units</th></tr>
<tr><td>Calcium</td><td></td><td colspan="2">mmol/mmol creat</td><td colspan="2">mg/mg creat</td><td>mmol/kg/24 h</td><td>mg/kg/24 h</td></tr>
<tr><td></td><td>0–1 year</td><td colspan="2"><2.29</td><td colspan="2"><0.81</td><td rowspan="7">All ages: <0.1 mmol/kg/24 h</td><td rowspan="7">All ages: <4 mg/kg/24 h</td></tr>
<tr><td></td><td>1–2 years</td><td colspan="2"><1.58</td><td colspan="2"><0.56</td></tr>
<tr><td></td><td>2–3 years</td><td colspan="2"><1.41</td><td colspan="2"><0.50</td></tr>
<tr><td></td><td>3–5 years</td><td colspan="2"><1.16</td><td colspan="2"><0.41</td></tr>
<tr><td></td><td>5–7 years</td><td colspan="2"><0.85</td><td colspan="2"><0.30</td></tr>
<tr><td></td><td>7–10 years</td><td colspan="2"><0.71</td><td colspan="2"><0.25</td></tr>
<tr><td></td><td>10–17 years</td><td colspan="2"><0.68</td><td colspan="2"><0.24</td></tr>
<tr><td>Citrate</td><td></td><td colspan="2">mmol/mmol creat</td><td colspan="2">mg/mg creat</td><td>mmol/1.73 m^2/24 h</td><td>mg/1.73 m^2/24 h</td></tr>
<tr><td></td><td>0–5 years</td><td colspan="2">>0.25</td><td colspan="2">>0.42</td><td>Boys – all ages: >1.9</td><td>Boys – all ages: >365</td></tr>
<tr><td></td><td>5+ year</td><td colspan="2">>0.15</td><td colspan="2">>0.25</td><td>Girls – all ages: >1.6</td><td>Girls – all ages: >310</td></tr>
<tr><td>Calcium-to-citrate ratio</td><td></td><td colspan="2">mmol calcium/mmol citrate</td><td colspan="2">mg calcium/mg citrate</td><td></td><td></td></tr>
<tr><td></td><td></td><td>Boys</td><td>Girls</td><td>Boys</td><td>Girls</td><td></td><td></td></tr>
<tr><td></td><td>2–6 years</td><td><0.75</td><td><0.65</td><td><0.15</td><td><0.13</td><td></td><td></td></tr>
<tr><td></td><td>7–12 years</td><td><0.85</td><td><0.60</td><td><0.17</td><td><0.12</td><td></td><td></td></tr>
<tr><td></td><td>13+ years</td><td><01.10</td><td><0.80</td><td><0.22</td><td><0.16</td><td></td><td></td></tr>
<tr><td>Creatinine</td><td></td><td colspan="2"></td><td colspan="2"></td><td>μmol/kg/24 h</td><td>mg/kg/24 h</td></tr>
<tr><td></td><td>Pre-pubertal</td><td colspan="2"></td><td colspan="2"></td><td>88–130</td><td>10–15</td></tr>
<tr><td></td><td>Pubertal boys</td><td colspan="2"></td><td colspan="2"></td><td>160–210</td><td>18–24</td></tr>
<tr><td></td><td>Pubertal girls</td><td colspan="2"></td><td colspan="2"></td><td>130–180</td><td>15–20</td></tr>
<tr><td>Cystine</td><td></td><td colspan="2">mmol/mmol creat and mg/g creat</td><td colspan="2">mg/mg creat</td><td>mmol/1.73 m^2/24 h</td><td>mg/1.73 m^2/24 h</td></tr>
<tr><td></td><td><1 month</td><td colspan="2"><85</td><td colspan="2"><180</td><td><10 years: <55</td><td><10 years: <13</td></tr>
<tr><td></td><td>1–6 months</td><td colspan="2"><53</td><td colspan="2"><112</td><td></td><td></td></tr>
<tr><td></td><td>6+ months</td><td colspan="2"><18</td><td colspan="2"><38</td><td>>10 years: <200</td><td>>10 years: <48</td></tr>
<tr><td>Magnesium</td><td></td><td colspan="2">mmol/mol creat</td><td colspan="2">mg/g creat</td><td>mmol/1.73 m^2/24 h</td><td>mg/1.73 m^2/24 h</td></tr>
<tr><td></td><td>0–1 year</td><td colspan="2">>2.2</td><td colspan="2">>0.48</td><td>>2 years: >3.6</td><td>>2 years: >88</td></tr>
<tr><td></td><td>1–2 years</td><td colspan="2">>1.7</td><td colspan="2">>0.37</td><td></td><td></td></tr>
<tr><td></td><td>2–3 years</td><td colspan="2">>1.6</td><td colspan="2">>0.34</td><td></td><td></td></tr>
<tr><td></td><td>3–5 years</td><td colspan="2">>1.3</td><td colspan="2">>0.29</td><td></td><td></td></tr>
<tr><td></td><td>5–7 years</td><td colspan="2">>1.0</td><td colspan="2">>0.21</td><td></td><td></td></tr>
<tr><td></td><td>7–10 years</td><td colspan="2">>0.9</td><td colspan="2">>0.18</td><td></td><td></td></tr>
<tr><td></td><td>10–14 years</td><td colspan="2">>0.7</td><td colspan="2">>0.15</td><td></td><td></td></tr>
<tr><td></td><td>14–17 years</td><td colspan="2">>0.6</td><td colspan="2">>0.13</td><td></td><td></td></tr>
<tr><td>Oxalate</td><td></td><td colspan="2">mmol/mmol creat</td><td colspan="2">mg/mg creat</td><td>mmol/1.73 m^2/24 h</td><td>mg/1.73 m^2/24 h</td></tr>
<tr><td></td><td><6 months</td><td colspan="2"><0.37</td><td colspan="2"><0.29</td><td><0.5</td><td><52</td></tr>
</table>

(continued)

Table 2 (continued)

Urinary Solute	Age	Random				24-h			
		SI Units		Conventional Units		SI Units		Conventional Units	
	6 months–2 years	<0.26		<0.20					
	2–5 years	<0.14		<0.22					
	6–12 years	<0.08		<0.06					
	12+ years	<0.04		<0.03					
Phosphorus		mmol/mmol creat		mg/mg creat		mmol/1.73 m^2/24 h		mg/1.73 m^2/24 h	
		Boys	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**
	2–3 years	<6.8	<7.7	<1.86	<2.11	<41	<47	<1271	<1457
	3–4 years	<6.6	<7.5	<1.81	<2.06	<41	<45	<1271	<1395
	4–5 years	<6.4	<7.2	<1.75	<1.97	<41	<44	<1271	<1364
	5–6 years	<6.1	<6.9	<1.67	<1.89	<40	<43	<1240	<1333
	6–7 years	<5.9	<6.6	<1.62	<1.81	<40	<42	<1240	<1302
	7–8 years	<5.7	<6.3	<1.56	<1.73	<40	<41	<1240	<1271
	8–9 years	<5.5	<6.0	<1.51	<1.64	<40	<40	<1240	<1240
	9–10 years	<5.2	<5.8	<1.42	<1.59	<40	<39	<1240	<1209
	10–11 years	<5.0	<5.5	<1.37	<1.51	<40	<38	<1240	<1178
	11–12 years	<4.8	<5.2	<1.32	<1.42	<40	<36	<1240	<1116
	12–13 years	<4.6	<4.9	<1.26	<1.34	<40	<35	<1240	<1085
	13–14 years	<4.3	<4.6	<1.18	<1.26	<40	<34	<1240	<1054
	14–15 years	<4.1	<4.4	<1.12	<1.21	<39	<33	<1209	<1023
	15–16 years	<3.9	<4.1	<1.07	<1.12	<39	<32	<1209	<992
	16–17 years	<3.7	<3.8	<1.01	<1.04	<39	<31	<1209	<961
	17–18 years	<3.4	<3.5	<0.93	<0.96	<39	<30	<1209	<930
Potassium						mmol/kg/24 h		mEq/kg/24 h	
						>3		>3	
Sodium						mmol/kg/24 h		mEq/kg/24 h	
						<3		<3	
Uric Acid		mmol/mmol creat		mg/mg creat		μmol/kg/24 h		mg/kg/24 h	
	<1 year	<1.5		<2.2		<1 year: <70		<1 year: <13	
	1–3 years	<1.3		<1.9		1–5 years: <65		1–5 years: <11	
	3–5 years	<1.0		<1.5		>5 years: <55		>5 years: <9	
	5–10 years	<0.6		<0.9					
	10+ year	<0.4		<0.6					

phosphate leak stimulate the activation of vitamin D, which increases intestinal calcium absorption and urinary calcium excretion, promoting calcium stone formation [65]. Finally, a markedly increased dietary calcium intake can cause hypercalciuria. Studies have documented a strong genetic component to idiopathic hypercalciuria, which is transmitted as a complex trait [75].

Hyperoxaluria

Hyperoxaluria has been reported in 4–40% of childhood stone formers [11, 76–78] (Tables 2 and 3). Hyperoxaluria can be caused by increased dietary intake (dietary hyperoxaluria), over-absorption of oxalate in patients with fat malabsorption (enteric hyperoxaluria), and inherited

Table 3 Metabolic abnormalities contributing to urolithiasis

Metabolic Risk Factor	Monogenic Causes				Secondary Physiologic or Exogenous Causes
	Gene	OMIM	Product	Common Name	
2,8-Dihydroxyadeninuria	*APRT*	102600	Adenine phosphoribosyltransferase	APRT deficiency	
Hypercalciuria	*ADCY10*	605205	Soluble adenylyl cyclase	Absorptive hypercalciuria	Dietary sodium excess
	ATP6V1B1	192132	B1 subunit of H-ATPase	Distal RTA	Dietary calcium excess
	CASR	601199	Calcium sensing receptor	AD hypocalcemia with hypercalciuria, Bartter's syndrome, type V	Vitamin D excess
	CLCN5	300008	ClC-5	Dent disease, type 1	Ketogenic diet
	CLDN16	603959	Claudin 16	Familial hypomagnesemia with hypercalciuria and nephrocalcinosis	Loop diuretics
	CLDN19	610036	Claudin 19	Familial hypomagnesemia with hypercalciuria and nephrocalcinosis with ocular abnormalities	Immobilization
	CYP24A1	126065	24-hydroxylase	24-hydroxylase deficiency/vitamin D hypersensitivity	Metabolic acidosis
	OCRL	300535	OCRL-1	Dent disease, type 2/Oculocerebral syndrome of Lowe	Premature birth
	SLC4A1	109270	Anion exchanger – 1	Distal RTA	Hypercalcemia
	SLC34A1	182309	NaPi2a	Hypophosphatemic nephrolithiasis/osteoporosis type 1	Hyperparathyroidism
	SLC34A3	609826	NaPi2c	Hereditary hypophosphatemic rickets with hypercalciuria	Hyper- or hypothyroidism
Hypercystinuria	*SLC3A1*	104614	rBAT amino acid transporter	Cystinuria	N/A
	SLC7A9	604144	b(0,+)-type amino acid transporter 1	Cystinuria	
Hyperoxaluria	*AGXT*	604285	Alanine:glyoxylate aminotransferase	Primary hyperoxaluria type 1	Dietary oxalate excess
	GRHPR	604296	Glyoxylate reductase/hydroxypyruvate reductase	Primary hyperoxaluria type 2	Enteric hyperoxaluria
	HOGA1	613597	4-hydroxy-2-oxoglutarate aldolase	Primary hyperoxaluria type 3	Gastric bypass surgery
	SLC26A1	610130	Oxalate transporter		Orlistat
	SLC26A6	610068	Oxalate transporter		Ethylene glycol

(continued)

Table 3 (continued)

Metabolic Risk Factor	Monogenic Causes				Secondary Physiologic or Exogenous Causes
	Gene	OMIM	Product	Common Name	
Hyperphosphaturia	*SLC34A1*	182309	NaPi2a	Hypophosphatemic nephrolithiasis/ osteoporosis type 1	Dietary phosphorus excess
	SLC34A3	609826	NaPi2c	Hereditary hypophosphatemic rickets with hypercalciuria	Hyperparathyroidism
Hyperuricosuria	*G6PC*	613742	Glucose 6-phosphatase	Glycogen storage disease type I	High animal protein intake
	HPRT1	308000	Hypoxanthine-guanine phosphoribosyltransferase	Lesch-Nyhan syndrome	Ketogenic diet
					Diabetes mellitus
					Tumor lysis syndrome
					High-dose pancreatic enzyme therapy
					Sulfinpyrazone
					Phenylbutazone
					Metabolic acidosis
Hypocitraturia	*ATP6V1B1*	192132	B1 subunit of H-ATPase	Distal RTA	Ketogenic diet
	SLC4A1	109270	Anion exchanger – 1	Distal RTA	Metabolic acidosis
					Hypokalemia
					Bacteriuria
					Massive blood transfusion

enzyme deficiencies [46, 55, 70]. Oxalate is an end product of metabolism that is eliminated by the kidneys [79]; even moderate changes in urinary oxalate excretion can significantly increase calcium oxalate supersaturation, promoting stone formation [79]. A substantial proportion of urinary oxalate is produced by the endogenous metabolism of glycine, glycolate, hydroxyproline, and by the metabolic degradation of dietary ascorbic acid [23]. Excessive dietary oxalate intake (rhubarb, spinach, celery, dark roughage, chocolate, cocoa, coffee and tea) also increases the urinary oxalate load [80] and the risk of calcium oxalate stone formation [81]. Dietary oxalate restriction in adult kidney stone formers with mild hyperoxaluria has been shown to decrease renal oxalate excretion by 35% [82]. In addition, intestinal oxalate absorption varies inversely to dietary calcium intake; calcium-poor diets promote intestinal oxalate absorption and hyperoxaluria [42]. By a similar mechanism, malabsorbed fatty acids in enteric hyperoxaluria bind intestinal calcium, increasing oxalate absorption [83]. Inflammatory bowel disease [84], celiac disease, cystic fibrosis (CF), other forms of exocrine pancreatic insufficiency, diseases of the biliary tract, and short bowel syndrome, are conditions associated with enteric hyperoxaluria [42].

Overall, 5–10% of patients with CF develop calcium oxalate stones [44]. In addition to hyperoxaluria [44, 85] secondary to pancreatic insufficiency, patients with CF often have reduced intestinal colonization with oxalate-degrading bacteria [44, 85] and hypocitraturia [85].

Bariatric surgery is a risk factor for kidney stone formation [86] that is primarily caused by malabsorptive enteric hyperoxaluria, and may lead to CKD [87, 88].

Hypocitraturia

Urinary citrate is a small molecular inhibitor of kidney stone formation [89]. Hypocitraturia has been reported in 15–70% of pediatric stone formers in various countries (Table 2) [11, 76, 78, 89]. Decreased citrate excretion may occur as an isolated abnormality or can be associated with other risk factors for stone formation. Citrate binds free urinary calcium, lowering calcium supersaturation [90] and augments the inhibitory effect of Tamm-Horsfall protein on calcium oxalate crystallization [91]. Hypocitraturia can be idiopathic [90] or is associated with metabolic acidosis [55, 70]. Intracellular acidosis in renal tubular cells secondary to systemic acidosis or hypokalemia increases citrate reabsorption, leading to hypocitraturia [42]. Idiopathic hypocitraturia can also be inherited [92]. Animal proteins increase the urinary acid load, which reduces urinary citrate concentration in addition to promoting skeletal calcium losses and hypercalciuria (see above) [70, 71]. In contrast, fruits and vegetables are rich in citrate, which is metabolized into carbon dioxide and water, increasing urinary pH [81].

Hyperuricosuria

Hyperuricosuria has been reported in 4–27% of pediatric stone formers [76], and is a risk factor for both calcium oxalate [93, 94] and uric acid stone formation (Table 2) [95]. High urinary uric acid concentration promotes calcium oxalate stone formation through heterogeneous nucleation [55]. Allopurinol reduces uric acid production and decreases the risk of stone formation in adults with idiopathic calcium nephrolithiasis [96].

The diagnosis of a uric acid stone in a child should always prompt physicians to look for inherited [46] or secondary causes of hyperuricosuria, including hypoxanthine-guanine phosphoribosyl-transferase (HPRT) deficiency or conditions associated with high uric acid production, such as myelo- and lymphoproliferative disorders, tumor lysis syndrome or ketogenic diet [44].

Structural Malformations of the Urinary Tract and Urinary Tract Infections

Structural malformations of the urinary tract may disrupt urine flow, and represent important risk factors for both stone formation and urinary tract infections. They have been reported in up to -one-third of pediatric stone formers [5, 11, 76]. In the past, a high proportion of urinary stones were associated with urinary tract abnormalities and infections caused by urease producing bacteria, leading to the formation of struvite stones, particularly in young boys [97]. Nowadays, infection-related urinary stones in children are becoming increasingly rare because antenatal ultrasound detects most structural urinary tract malformations, allowing early interventions that reduce the risk of infections [97]. Splitting of urea results in the production of ammonium and bicarbonate ions, which can increase urine pH to 7 or higher [98]. Alkaline pH dissociates phosphate, causing precipitation of calcium phosphate and magnesium ammonium phosphate (struvite) that aggregate on biofilms produced by bacteria. Calculi then grow rapidly with the addition of more bacteria, with the production of more matrix, and with the addition of new crystals, leading to the formation of staghorn calculi [99]. When present, struvite stones should always be completely removed because residual fragments host bacteria and serve as nidi for new stone formation [98, 100]. The eradication of infection is particularly difficult because antibiotics penetrate poorly into stones, where biofilms hosting bacteria develop [44]. At least 200 bacterial species are capable of producing urease, including *Proteus, Providentia, Haemophilus influenzea, Bordatella pertussis, Staphylococcus, Klebsiella, Pseudomonas, Enterobacter, Ureaplasma urealyticum, Corynebacterium urealyticum*, and some anaerobes [44, 98]. Struvite stones have been associated with significant morbidity including acute kidney injury (AKI), CKD, xanthogranulomatous pyelonephritis (chronic granulomatous inflammation of the kidney), and even mortality, if not properly treated [44, 98]. Since extracorporeal shockwave lithotripsy (ESWL) allows complete fragmentation and clearance of staghorn struvite calculi in less than 50% of cases [44], percutaneous nephrolithotomy is usually the method of choice for treating these stones. Treatment with acetohydroxamic acid reduces struvite stone recurrence rate; nowadays, this treatment is only prescribed if endourological treatment is not possible or when complete stone removal has not been successful [44, 98]. Patients should always be treated with antibiotics to eradicate infections; stone fragments and urine collected from the renal pelvis during surgery should be cultured before initiating a new course of antibiotics because midstream urine cultures do not always reveal the causative organism [100]. Prophylactic antibiotic treatment is frequently prescribed for 3–6 months following surgery to reduce the risk of recurrence [98]. Patients with struvite stones often have other urine metabolic risk factors for urinary stone disease [44], although their relevance is uncertain [98, 101].

Ureteropelvic junction obstruction, calyceal diverticuli, medullary sponge kidney, horseshoe kidney, ureteroceles, primary megaureter, posterior urethral valves, and bladder extrophy-epispadias complex are the congenital urinary tract disorders most commonly associated with urolithiasis [44]. Patients with spinal cord injury are at higher risk of kidney stone formation as they often have impaired bladder emptying causing recurrent urinary tract infections, and they develop hypercalciuria secondary to immobilization [44].

Drug- and Toxin-Induced Urinary Stones

Several medications and toxins can cause urinary stone formation in children and adults. Drug-induced kidney stones account for approximately 1% of calculi [102]. Stones may form by two mechanisms: (a) the stones can be directly composed of the drug or its metabolites or (b) the drug may cause metabolic abnormalities leading to the formation of calcium and less frequently, uric acid stones [102]. Examples of the first mechanism include calculi associated with protease inhibitors (indinavir, atazanavir, nelfinavir and tenovir), sulfonamides (sulfadiazine, sulfazalazine, sulfamethoxazole), antibiotics (ceftriaxone, ampicillin, amoxicillin) and antiviral agents (efavirenz), triamterene, many over-the-counter cough suppressants, expectorants (guaifenesin) or decongestants (ephedrine, pseudoephedrine), allopurinol, or silica-containing antacids; these drugs or their metabolites can crystalize in urine causing stone formation [42, 102]. Stones containing drugs are typically radiolucent and can be detected by ultrasound or non-contrast helical CT [102]. Indinavir stones can be particularly challenging to visualize [103].

Examples of drugs that induce metabolic alterations that promote stone formation include carbonic anhydrase inhibitors (acetazolamide, topiramate, zonisamide), calcium supplements, vitamin D, furosemide, or corticosteroids [41, 104]. Topiramate and other carbonic anhydrase inhibitors block proximal tubular reabsorption of sodium and bicarbonate, causing intracellular acidosis and increased citrate reabsorption, leading to urinary alkalinization, hypocitraturia, and hypercalciuria, all of which promote the formation of calcium phosphate stones [102, 105]. Uricosuric drugs increase the risk of uric acid stones, while allopurinol increases the risk of xanthine and oxypurinol stones.

Since drug-induced stones are often asymptomatic, a high index of suspicion and close monitoring of patients at-risk is necessary for timely diagnosis and treatment [104]. In the differential diagnosis of drug-induced stones, other radiolucent stones should also be considered, including 2,8-dihydroxyadenine (DHA), uric acid and, xanthine and sometimes, cystine stones. Conversely, stones secondary to treatments that modify urine composition can be distinguished from idiopathic calcium phosphate or calcium oxalate stones only with a thorough medical history.

When possible, the drugs involved should be discontinued. If alternative therapies are not available, manipulation of urine pH and volume may help preventing new stone formation or stone growth. ESWL is an effective therapy for most drug-induced stones [102].

In 2008, at least 52,000 Chinese babies were hospitalized with melamine-containing radiolucent kidney stones related to the consumption of melamine-tainted infant formula [106, 107]. Some children developed AKI secondary to urinary obstruction. After four years of follow-up kidney stones had resolved in most cases with conservative treatment only [107].

Clinical Conditions Associated with Urinary Stone Disease

Urinary stone disease is a common symptom of several inherited metabolic disorders that are detailed elsewhere in the textbook (see ▶ Chaps. 30, “The Kidney in Metabolic Syndrome,” and ▶ 38, “Renal Tubular Acidosis”) [108].

In addition, several clinical conditions increase the risk of urinary stone disease [44] and have in part been discussed in the above paragraph. They include the use of some medications, disorders that alter the acid-base equilibrium, neurological and malabsorptive conditions, prematurity, and renal parenchymal diseases, among others [44, 105]. Children with severe neurological disorders are at particularly high risk of developing urinary stones [104] because they are frequently treated with anti-epileptic drugs or with ketogenic diets, they are often immobilized [109–111], and their fluid intake may be insufficient.

Renal parenchymal disorders may be complicated with urinary infections and stones [44]. Medullary sponge kidney (MSK) is associated with recurrent calcium oxalate and/or phosphate stone disease [112]. Distal renal tubular acidosis (dRTA) has been reported in a significant proportion of affected patients, and hypercalciuria is present in nearly 50% of cases [112, 113]. Treatment with potassium citrate reduces stone recurrence in MSK.

Kidney stones have also been reported in 25–30% of patients with autosomal dominant polycystic kidney disease (ADPKD) [44, 114, 115], including uric acid (57%), calcium oxalate (47%), calcium phosphate (20%), and struvite (10%) stones [114]. Risk factors for stone formation in ADPKD include hypocitraturia, hyperuricosuria, hyperoxaluria, and hypercalciuria [114, 115]. Tolvaptan therapy significantly improves the urinary lithogenic risk profile in patients with ADPKD [116].

Increased frequency of urinary stones has also been described in patients with thin basement membrane disease [117].

Mechanism of Stone Formation

The pathogenesis of urinary stone disease in humans is complex and involves the interaction of genetic and environmental factors that vary according to the type of stone [118]. Kidney stones are thought to develop on structural lesions, driven by the urinary supersaturation of solutes. In 1937, Randall described renal papillary lesions which today are referred to as Randall's plaques and plugs; he hypothesized that stones only form and grow if attached to these lesions [119]. Currently, urinary calculi are thought to form by one of three major mechanisms [118]. First, kidney stones can develop on preexisting subepithelial interstitial plaques of calcium phosphate (Randall's plaques) that are present in the renal papillae. The second mechanism involves the deposition of crystals in renal tubules (Randall's plugs) that are primarily located in the inner medullary collecting ducts and in the ducts of Bellini (BD). Finally, stones can form in the fluid phase after crystallization of salts [118, 120].

The first pathway involves the formation of interstitial apatite or calcium phosphate Randall's plaques that probably originate in the basement membranes of the thin loops of Henle, from where they spread to the suburothelial space [121]. Randall's plaques are not associated with renal tissue damage [121]. Stones only form if plaques erode the papillary surface of the urothelium; once exposed, stone formation starts, driven by the urinary supersaturation of the stone forming salts [118]. Randall's plaques are very powerful triggers of stone formation, as indicated by the large number and size of calculi that grow attached to the small area of papillary surfaces (3.5–7.5%) covered with plaques [122, 123]. The plaque abundance on the papillary surface is associated with the number of urinary stones [124] and urinary calcium excretion, and is inversely associated with urinary volume and pH in idiopathic calcium stone formers, in obese patients that have undergone bariatric surgery, and in healthy individuals [123]. Since plaques form within the renal papilla, they are not thought to develop as a direct result of abnormal urinary supersaturating conditions, but idiopathic hypercalciuria, urinary pH, and urine concentration probably play a role [118, 123]. Hypothetically, increased water reabsorption increases calcium concentration along the loop of Henle, while urinary acidification raises pH in the papillary interstitium, favoring calcium phosphate deposition [118]. The thin limb basement membrane, which is comprised of negatively charged collagen and mucopolysaccharide molecules, attracts both calcium and phosphate through electrostatic forces [121]. In addition, the development of Randall's plaques depends on the stone type. It is present in nearly 100% of calcium and uric acid stones, 30% of cystine stones, and 20% of struvite stones [125]. Since idiopathic calcium stones are the most common type of urolithiasis, growth on Randall's plaque is the primary mechanism of stone formation in humans [118, 126]. Plaques are also observed in subjects without history of kidney stones [125], but tend to be larger in stone formers [121]. It is unclear at what age Randall's plaque begins to form; to date, no studies have analyzed Randall's plaque formation in the pediatric population [16].

Randall's plugs form when stone-forming salts precipitate and clog collecting ducts, protruding into the renal pelvis where they trigger stone formation and growth [118]. Plugging in the renal tubular lumen is driven by the urinary supersaturation of lithogenic factors and is generally associated with tubular epithelial injury and inflammation [120], contrary to Randall's plaques [121]. Different types of stones, including brushite, calcium oxalate, cystine, and calcium phosphate stones, have been found attached to Randall's plugs [120]. Plaques and plugs may coexist in patients with different types of stones, including patients with idiopathic calcium oxalate calculi [122, 127]. Linnes et al. [122] have shown that 99% of stone formers have Randall's plaques, but approximately 20% of patients also have tubular plugging, indicating that tubular plugging is more common than previously recognized and that the two mechanisms coexists in a significant proportion of patients [122].

The last mechanism of stone formation involves crystallization in the fluid phase of poorly soluble stone-forming molecules, such as cystine [118, 128]. Other highly insoluble salts, including DHA and xanthine, are likely to form stones by the same mechanism, although the exact pathophysiology has not yet been elucidated. Once anchored to a lesion, stone growth is driven by supersaturating conditions that are specific to the different stone types [63, 118].

The solubility or saturation point for the different stone-forming minerals or ions is the point of equilibrium at which crystals in solution neither grow nor dissolve [63]. A solution is undersaturated when crystals dissolve and supersaturated when crystals grow [55]. Urinary supersaturation values have been found to correlate with stone composition [129]. Urinary supersaturation is multifactorial, including concentration of calcium, oxalate, phosphate and sodium, urinary osmolality, urine pH, and complexation of stone-forming constituents with other molecules (e.g., magnesium, citrate, and urinary proteins) [63]. Acidic urine favors cystine (pH <7.5) and uric acid (pH <6.0) crystallization, while calcium phosphate stones form in more alkaline urine (pH >6) [42]. Calcium oxalate crystallization is not significantly influenced by pH in the physiologic range. Homogeneous nucleation (i.e., spontaneous formation of crystals in an oversaturated solution) is much less common than heterogeneous nucleation (i.e., formation of crystals on Randall's plaques or plugs or other types of crystals) [55, 63]. The human urine inhibits aggregation, nucleation, adhesion, and growth of calcium oxalate monohydrate crystals [130]. This explains why urinary calcium oxalate concentration is frequently above the solubility or saturation threshold, and crystals only form when the supersaturation reaches 7–11 times the solubility [55]. Interestingly, inhibition of calcium oxalate monohydrate crystal formation is inversely related to age; the urine of pediatric patients inhibits crystallization more effectively than adult urine [131]. This may, at least in part, explain why children form stones less frequently than adults [130]. Several proteins acting as crystallization inhibitors have been identified; of these, uropontin [132], nephrocalcin [133], and Tamm-Horsfall protein, also termed uromodulin, are most significant [63, 134, 135]. Citrate, pyrophosphate, and magnesium are the main nonprotein urinary crystallization inhibitors [63].

Genetics of Urinary Stone Disease

Family Studies

A positive family history has been reported in 25–65% of subjects with urinary stone disease in adults [136–139] and 33% of children [11].

Twin Studies

One twin study based on data from the Vietnam Era Twin Registry has estimated the heritability of urinary stone disease to be 56% [140]. In the same study, the concordance rate in monozygotic twins (32.4%) was significantly greater than the rate in dizygotic twins (17.3%) ($P<0.001$), consistent with a genetic influence.

Genealogy Studies

Familial clustering of kidney stones extends beyond the nuclear family in population-based studies from Iceland [141, 142]. In patients with radiopaque urinary calculi, the relative risk of stones ranged from 2.25 ($p<0.001$) for first degree relatives to 1.07 ($p<0.01$) in fifth degree relatives. The relative risk of urinary stones in parents and offspring of recurrent stone formers was >10 ($p<0.001$) [141]. Icelandic kidney stone formers were also found to be genetically more related to each other compared to the average Icelanders. These data provide strong evidence in support of a genetic component in the risk of developing kidney stones, and mitigate the effect of environmental factors.

Genome-Wide Association Studies

Linkage and candidate gene studies have been disappointing in identifying genes predisposing to develop urinary stones. Conversely, genome-wide association studies have been more successful [143, 144]. A study of 3,773 cases and 42,510 controls in Iceland and the Netherlands identified synonymous *CLDN14* sequence variants that associate with kidney stones, reduced bone mineral density, lower serum bicarbonate levels, higher urinary calcium excretion, and higher parathyroid hormone levels [143]. Approximately 62% of the general population is homozygous for these variants and has 1.64 times greater risk of developing kidney stones compared to noncarriers. CLDN14 protein is a member of the claudin family of membrane proteins that regulate paracellular passage of ions and small solutes at epithelial tight junctions [145]. Increased risk of urinary stones associated with *CLDN14* variants may result from the combined effects of changes in serum bicarbonate, urinary calcium, and parathyroid hormone levels. A second genome-wide association study conducted in 3,617 Icelandic and Dutch cases and 43,201 controls discovered a variant in the uromodulin gene conferring protection against kidney stones [144]. Uromodulin is a well-known urinary inhibitor of calcium stones [146]; variants in the uromodulin gene may reduce the ability of the protein to complex with urinary calcium, resulting in an increased risk of stone formation.

Rare Monogenic Causes of Urinary Stone Disease

Monogenic disorders account for approximately 10% of urinary stone disease in children and adolescents and 2% in the adult population [147]. These rare disorders are characterized by more severe stone disease, compared to common polygenic forms of stones. In addition they are frequently characterized by the development of CKD, secondary, at least in part, to crystal nephropathy. Their main features are summarized in Table 3; most of these disorders are covered in other specific chapters of this textbook.

Recurrent kidney stones and/or nephrocalcinosis, particularly in younger children, should therefore alert physician to the possibility of an underlying genetic cause. The lack of recognition of these disorders has frequently resulted in unacceptable delay in both diagnosis and treatment, sometimes with grave consequences [46, 148]. Clinical features that suggest rare monogenic causes of kidney stones are summarized in Table 4 [46].

Clinical Features

The acute presentation of urinary stone events in children is highly variable. Colicky abdominal, flank, or lower back pain is the most common presenting symptom in older children, and is reported in 50–80% of cases [11, 16]. Nonspecific abdominal pain and irritability are more common in younger children and infants [9]. Nausea and vomiting are often present. Gross hematuria is the presenting sign in 30 to 50% of cases; microhematuria is nearly always present during acute episodes [11, 16]. Other frequent features include urinary tract infections, lower urinary tract symptoms, or urinary retention if stones obstruct the bladder outlet or the urethra [9]. In some inherited conditions, acute kidney injury episodes may be underdiagnosed [149, 150], and only recognized retrospectively after patients have developed CKD [46].

Diagnostic Evaluation

Thorough medical history and physical examination, coupled with laboratory investigations and imaging are essential for the clinical evaluation of children with kidney stones. Medical history should focus on dietary information, episodes of

Table 4 When to suspect rare causes of kidney stones [46]. (Modified with permission from Springer Science and Business Media)

History	First stone as a child Family history of stones or nephrocalcinosis or unexplained kidney failure Brownish-red diaper stain[a] Growth retardation or rickets
Laboratory testing	Crystals of unusual type Cystine[b] 2,8-Dihydroxyadenine[a] Unidentified Mild-moderate proteinuria[c] Elevated serum creatinine and/or reduced GFR Hypomagnesemia and increased urinary calcium excretion[d] Increased urinary oxalate excretion[e] Positive urinary sodium nitropusside test[f] Significant hypouricemia[g] Persistent hypercalcemia or hypophosphatemia
Imaging	Nephrocalcinosis Radiolucent kidney stones Multiple stones, bilateral stones

GFR, glomerular filtration rate
[a] Suggestive of adenine phosphoribosyltransferase deficiency
[b] Diagnostic of cystinuria
[c] Low molecular weight proteinuria is suggestive of Dent disease
[d] Highly suggestive of familial hypomagnesemia with hypercalciuria and nephrocalcinosis
[e] May suggest primary hyperoxaluria
[f] Suggestive of cystinuria in children 2 years and older
[g] Suggestive of hereditary xanthinuria or idiopathic renal hypouricemia

dehydration, immobilization, use of nutritional supplements such as vitamin C, vitamin D, and minerals, and medications [9, 42]. A family history of urinary stones, hematuria, kidney failure, and/or metabolic disorders may be very informative. Clinical evaluation should look for features associated with disorders of mineral and vitamin D metabolism, including bone deformities, growth retardation, and signs of rickets or tetany [9, 42].

All childhood stone formers require a thorough laboratory evaluation following the first urinary stone episode to identify modifiable risk factors and to diagnose rare causes of stones, some of which may be associated with CKD (renal parenchymal lesions, proteinuria, evidence of tubular dysfunction, etc.) [42, 46]. Urine culture should be obtained if a urinary tract infection is suspected. Urine microscopy looking for urinary crystals should be performed at least once in all patients with kidney stones [46]. The characteristic round and brown DHA crystals (Fig. 2a) and hexagonal cystine crystals (Fig. 2c) are easily detected by urine microscopy and are diagnostic of 2,8-dihydroxyadeninuria and cystinuria, respectively. Calcium oxalate dihydrate crystals (Fig. 2d) are present in the urine of patients with high oxalate excretion, though they are also commonly seen in patient with idiopathic calcium oxalate stone disease and in non-stone forming subjects with concentrated urines. Patients with PH often have calcium oxalate monohydrate crystals in their urine, while in individuals with Dent disease, calcium oxalate or calcium phosphate crystals may be noted (Fig. 2e) [46]. Whenever stone fragments are available, their composition should be analyzed. The analysis of stone composition is diagnostic when calculi contain cystine, DHA, xanthine, and struvite [9, 42, 46]. Stone fragments should be collected by straining urine for several days following lithotripsy or a symptomatic stone event [105]. Although nonspecific, the finding of a calcium-based stone helps narrow the differential diagnosis and guides therapeutic approach. Since idiopathic uric acid stones are extremely rare in children, all pediatric uric acid stone

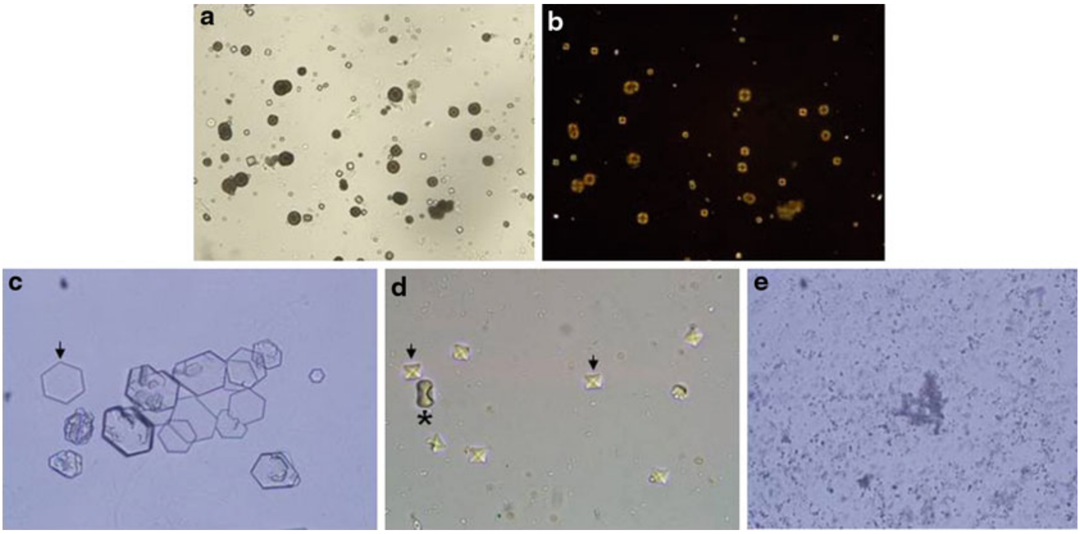

Fig. 2 Urinary crystals (Original magnification × 400). (**a**) The typical small and medium sized 2,8-dihydroxyadenine crystals. The medium sized crystals are brown with dark outline and central spicules. (**b**) The same field viewed with polarized light microscopy. The small and medium sized crystals appear yellow in color and produce a central Maltese cross pattern. (**c**) The typical six-sided cystine crystals are diagnostic of cystinuria. A good example can be seen on the left side of the Figure (arrow). (**d**) Urinary calcium oxalate crystals. The typical bipyramidal calcium oxalate dihydrate crystals (arrows) and a large dumbbell calcium oxalate monohydrate crystal (asterisk) are both seen. (**e**) Amorphous calcium phosphate crystals. Although their appearance is not as distinctive, amorphous calcium phosphate crystals should be suspected if the urine is alkaline (pH>6.0). (Reproduced with permission from Springer Science and Business Media)

formers should be screened for inborn errors of purine metabolism or renal tubular uric acid transport defects [46].

Comprehensive evaluation of urinary stone disease should also include serum electrolytes, bicarbonate, creatinine, blood urea nitrogen, and uric acid levels [105, 151]. Measuring serum concentrations of PTH, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D should also be considered. The 24 h urine excretion of calcium, oxalate, citrate, uric acid, sodium, and creatinine, as well as urinary volume and pH, should be measured, and, if possible, supersaturation profiles for calcium oxalate, calcium phosphate, and uric acid should be obtained after a first stone episode [9, 63, 152]. Normal values for urinary solute excretion in children are detailed in Table 2. In addition to measuring calcium and citrate excretion, assessing urinary calcium-to-citrate ratio can be very helpful for predicting stone formation and recurrence (Table 2) [153, 154]. Although 24 h samples are preferable for most urine parameters, random urine samples can be used in children who are not toilet trained. Since urinary solute excretion rates may vary significantly, repeat urine collections are frequently needed to confirm results.

Inherited metabolic disorders such as APRT deficiency, cystinuria, Dent disease, familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC), PH, and other rare disorders should be considered in the differential diagnosis of children with kidney stone disease and/or CKD, particularly when stones develop early, are multiple, and are bilateral [46]. Cystinuria should be ruled out with a qualitative (sodium nitroprusside test) or quantitative urinary cystine measurements [46]. LMW proteinuria should be ruled out in all boys with unexplained stones, nephrocalcinosis, and proteinuria, to exclude Dent disease [46]. Hypomagnesemia and hypercalciuria are strongly suggestive of FHHNC. In the absence of conditions causing fat malabsorption, hyperoxaluria should raise the suspicion of PH. Parathyroid hormone and vitamin D levels should be measured in patients with abnormal serum calcium and/or phosphate concentrations [46, 105].

In adults, non-contrast CT is the gold standard for imaging urinary stones [105]. Caution should be used when exposing children to ionizing radiations [151]. The American Urological Association and the European Association of Urology have developed separate guidelines for the imaging approach of children with urinary stone disease [151]. Both associations recommend using ultrasound as the first-line approach, and to use non-contrast CT only in children with a negative ultrasound but a high suspicion of urolithiasis [151]. Although ultrasound is less sensitive and less specific than CT [155, 156], it accurately identifies most clinically significant urinary stones in the pediatric population. In a cohort of 50 pediatric patients, the sensitivity and specificity of the ultrasound were 70% and 100%, respectively, compared to CT (gold standard), and the positive and negative predictive values were 96% and 62%, respectively [156].

Management of Acute Urinary Stone Events

Children with acute symptomatic stone events have pain, often have nausea and vomiting, and some may become dehydrated, all of which require prompt intervention [105]. Intravenous ketorolac is more effective than intravenous opioids for acute pain relief [157, 158]. In adults with renal colic, 56% of patients receiving ketorolac and 80% of patients receiving meperidine required repeat analgesia within 24 h in a prospective double blinded randomized study [159]. In children, several studies have documented that ketorolac is safe and effective for pain management in other conditions [160–162]. For example, one study compared 0.75, 1.0, and 1.5 mg/kg of intravenous ketorolac with 0.1 mg/kg of intravenous morphine immediately prior to dental surgery [163]. Ketoralac controlled pain as effectively as morphine and was associated with significantly less postoperative vomiting. Indirectly, these studies indicate that ketorolac is a valid first-line agent for pain management in children with a renal colic, while other analgesics, including acetaminophen and opioids, can be added if needed [105].

Stones larger than 5–10 mm in largest diameter frequently require surgical intervention. Early consultation with a pediatric urologist should be obtained in children with large stones. Different stone removal techniques are available. Very young patients can be offered ESWL, or minimally invasive techniques, including retrograde ureteroscopic or percutaneous lithotripsy (PCNL) [44]. ESWL is still recommended for upper tract stones up to 15 mm in diameter (largest diameter), although a significant number of stones require repeated treatments [164], and there is risk for parenchymal damage with this technique [165]. Open lithotomy is rarely required and has been replaced with noninvasive or minimally invasive techniques, thanks to the development of the small and versatile endoscopic instruments [164].

Antiemetic treatment is frequently necessary in patients with nausea and vomiting associated with acute urinary obstruction [105]. The most commonly used agents in pediatrics are serotonin antagonists, namely ondansetron, promethazine, and metoclopramide [166]. Ondansetron is typically used in the acute setting, while oral promethazine is usually prescribed for outpatient management [105]. Restoration of euvolemia is needed if patients are dehydrated [42, 105]. This usually requires intravenous administration of isotonic fluids, because oral hydration is often not tolerated. Overhydration in the intent to facilitate stone passage is contraindicated because it can increase pain and the need for more analgesics [105].

Outpatient management of children with symptomatic urolithiasis is possible if patients can drink. Medical expulsive therapy comprises the use of alpha or calcium channel blockers to facilitate ureteral stone passage, and is frequently attempted to prevent surgical procedures [151]. These treatments are based on the high densities of α_{1a}, α_{1d}, and calcium channel receptors in smooth muscle cells of the distal ureter and ureterovesical junction that allow alpha and calcium channel blockers to dilate the ureter [151]. To date, three pediatric studies have tested the efficacy of medical expulsive therapy [167–169]. The first study was underpowered and found no differences in stone passage rates between doxazosin and a placebo [167]. The second study showed higher stone passages in children treated with tamsulosin compared to placebo, but results should be interpreted with caution because half of patients in the tamsulosin group had been treated with ESWL [168]. The third study [169] evaluated retrospectively the effect of tamsulosin on the passage of stones less than 10 mm in largest diameter. After adjusting for stone size and location, the odds for spontaneous stone passage were 3.3 times higher in treated compared to untreated children. Overall, the level of evidence supporting the use of tamsulosin for ureteral stones in children is moderate [151], and the efficacy of doxazosin is unclear. In addition, the benefits of combined therapies with medical expulsive therapy and anti-inflammatory drugs need to be studied in children.

Spontaneous passage of ureteral stones in children has been found to be remarkably similar across all ages for stones measuring less than 5 mm, while passage rates decrease very significantly for larger stones [170]. Spontaneous stone passage in children is also similar or even superior to that observed in adults [105]. Therefore, conservative treatment, including medical expulsive therapy, should be attempted for pediatric ureteral stones less than 4–5 mm in size, when infection is not suspected, pain control is adequate, and patients do not have AKI (single renal system, bilateral obstruction). Patients are typically given a minimum of 2 weeks to allow for spontaneous stone passage before surgical/endoscopic intervention is considered [105]. Emergency obstruction relief or aggressive antibiotic therapy is indicated in patients with complete urinary obstruction or severe urinary tract infection [105].

Prevention of Urinary Stone Recurrence

Children with urolithiasis are at high risk of recurrence [6, 7]. Ideally, they should be seen every 1–2 years in a stone clinic by a dedicated medical team [105]. Patients with known metabolic risks and those who have relapsing stones need to be followed more frequently [44]. Patients who form new stones or have stones that increase in volume despite treatment need intensification of their therapy [44]. A full 24 h urine stone risk factor evaluation and assessment of kidney function should be performed at routine follow-up visits. Other tests must be tailored to individual needs. Ultrasound is the preferred imaging modality to assess changes in stone size [44]. The goal of medical treatment is to reduce urine supersaturation of stone-forming salts or minerals. A comprehensive treatment plan must include recommendations for (a) minimum fluid intake; (b) appropriate dietary changes; and (c) pharmacological treatment.

Fluid Intake

High fluid intake is the most important treatment for all patients with kidney stones. The minimum recommended intake in children with the common stone types is 1.5–2 L/m^2/day [42, 51]. During warm weather and physical exercise, fluid intake must be increased. Patients with hereditary metabolic conditions such as PH or cystinuria need larger urine output to adequately reduce urinary concentration and supersaturation of the respective stone forming salts (infants:>750 mL/24 h; 1–5 years: >1,000 mL/24 h; 5–10 years: >1,500 mL/24 h; older children and adolescents: >2,000 mL/24 h; older adolescents and young adults: >3,000 mL/24 h) [46]. Gastric tube placement may be necessary to achieve adequate hydration in very severe cases. The risk of stone recurrence is probably increased by the excessive consumption of sugary beverages [151] because fructose- and glucose-containing drinks increase urinary excretion of calcium and oxalate [171]; these should be avoided in stone formers [151]. Children with calcium stones may benefit from beverages rich in citrate such as lemonade and orange juice, although these drinks tend to contain considerable amounts of sugar [42].

Dietary Considerations

Quality studies of dietary interventions in pediatric stone disease have not been performed

[151]. Since sodium is known to increase urinary calcium excretion, limitation of sodium intake to 2–3 mEq/kg/day is recommended [42, 51]. Monitoring urinary sodium-to-potassium ratios may be useful to provide feedback when decreasing sodium intake and promoting potassium-rich foods [172]. Excessive animal protein consumption should be avoided [42]. Dietary purine intake parallels animal protein intake, and increases uric acid excretion in urines [151]. The recommended protein intake of children with urinary stone disease should be close to 100% of the recommended daily allowance (RDA) to ensure growth and development [42]. Children with acidic urine or hypocitraturia benefit from diets rich in fruits and vegetables [81]. The contribution of diet to urinary oxalate excretion may be significant [80]. Therefore, children with calcium oxalate stones and/or hyperoxaluria should avoid foods rich in oxalate (beets, chives, chocolate, green tea, nuts, okra, parsley, rhubarb, soy beans, spinach, star fruit, sweet potatoes, tofu, and wheat bran) and vitamin C supplements [42, 51, 105]. The optimal calcium intake in children and adolescents with urinary stone disease is not known. The risk of stone recurrence in prospective observational studies has been inversely associated with dietary calcium intake [173]. Low calcium diets do not prevent calcium stones and carry the risk of inducing hyperparathyroidism and compromising bone health; in stone formers, these diets offer no advantages over normal calcium diets with reduced animal protein and sodium content [24]. Children with urinary stones should be allowed normal dietary calcium in line with the RDA, but excessive calcium intake should be avoided [42].

Pharmacological Treatment

Pharmacological therapy is indicated in children with recurrent calcium-based stones or with treatable hereditary disorders causing urolithiasis (Table 5) [42, 51]. The goal of treatment is to reduce urinary concentration and supersaturation of stone-forming compounds. Urinary supersaturation can be lowered by (a) reducing the excretion of the stone-forming compounds; (b) complexation with inhibitors of crystal formation; and (c) manipulation of urinary pH.

The pharmacological treatment of patients with calcium stones and hypercalciuria includes thiazide diuretics that reduce renal calcium excretion [51]. This treatment should be limited to the most severe cases. Hydrochlorothiazide is the most commonly used drug, and the recommended dose is 0.5–2 mg/kg/day in children, given twice a day [42, 51]. The longer-acting thiazide compound chlorthalidone can be prescribed once per day, which may improve compliance. Monitoring of serum electrolytes should be performed, especially in young patients. Amiloride can be added to thiazides

Table 5 Treatment for metabolic stone disease [44]. (Modified with permission from Dawn Milliner, MD, Mayo Clinic, Rochester Minnesota and Springer Science and Business Media)

Metabolic abnormality	Initial Treatment	Second line Treatment
Hypercalciuria	Reduce dietary Na^+ Dietary calcium at RDA Thiazides[a]	Potassium citrate Thiazides Neutral phosphate
Hyperoxaluria	Reduce dietary oxalate Potassium citrate	Neutral phosphate Magnesium Pyridoxine[b]
Hypocitric aciduria	Potassium citrate	Bicarbonate
Hyperuricosuria	Alkalinization (K-citrate)	Allopurinol
Cystinuria	Alkalinization Reduce dietary Na^+	Thiola D-penicillamine Captopril

[a] If hypercalciuria is severe or there is osteopenia
[b] In pyridoxine responsive primary hyperoxaluria

in patients who develop hypokalemia and hypocitraturia, or if the hypocalciuric effect of thiazide treatment is suboptimal [105]. No pediatric studies have assessed the benefits of thiazides on stone recurrence. Randomized trials in adults have shown a 40–80% reduction in the stone recurrence rate of treated patients [151]. Caution should be used in combining thiazide treatment with potassium citrate supplementation; while these compounds compensate their effect on potassium, they both increase serum bicarbonate levels and may cause severe alkalosis.

Oral potassium citrate should be prescribed to children with calcium stones and hypocitraturia at doses of 2–4 mEq/kg/day (30–90 mEq/day in adults) [42]. Citrate binds to urinary calcium to form a soluble complex which decreases urinary calcium oxalate supersaturation; in addition, it directly inhibits crystal growth and aggregation [151, 174]. Citrate significantly increases the urinary alkali load. Therefore, care should be exercised preventing urinary pH to rise above 6.3–6.5 because the risk of calcium phosphate stone formation increases in alkaline urine [42, 151]. Clinical trials in adults have demonstrated a reduction in stone recurrence in patients treated with potassium citrate, while the evidence is less robust in children [151].

Children with urinary stones secondary to dRTA have chronic acidemia, hypercalciuria, hypocitraturia, and frequently form calcium phosphate stones [151]. The recommended treatment in this condition is potassium citrate in daily doses of 2–4 mEq/kg, which have been shown to normalize urinary calcium and citrate excretion, as well as serum bicarbonate levels [175]. The urine pH of these patients tends to be be high because of impaired proton excretion and alkali leak; urine pH should be monitored to prevent increasing urine pH above 6.3–6.5 [42, 151].

Children treated with ketogenic diets are at increased risk for kidney stones because they develop metabolic acidosis, hypercalciuria, hypocitraturia, and usually have low fluid intake; in contrast to children with dRTA, they have low urine pH [44, 151]. Stone composition includes uric acid, calcium phosphate, mixed calcium and uric acid, and ammonium urate [44]. Potassium citrate reduces the incidence of urinary stones in these patients; prophylactic potassium citrate treatment has been proposed during ketogenic diet [176].

Treatment of children with pure uric acid stones includes alkali therapy aiming at urinary pH not exceeding 6.5, to avoid the risk of calcium phosphate stone formation. These patients tend to have low urine pH and are best treated with potassium citrate with average doses of 1–2 mEq/kg/day divided in two daily doses [151].

Oral administration of neutral phosphate and magnesium preparations, which bind urinary calcium and oxalate, respectively, may reduce the risk of forming stones in selected patients.

Treatment of hereditary monogenic causes of kidney stones (APRT deficiency, cystinuria, Dent disease, PH) is described in other chapters of this textbook.

Cross-References

- ▶ Aminoaciduria and Glycosuria in Children
- ▶ Hyperoxaluria
- ▶ Inherited Disorders of Renal Calcium Handling
- ▶ Inherited Disorders of Renal Magnesium Handling
- ▶ Renal Manifestations of Metabolic Disorders in Children
- ▶ Renal Tubular Acidosis

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Urinary Tract Infections in Children

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Abstract

Urinary tract infection (UTI) remains among the most common bacterial infections in childhood. An association between UTIs and abnormalities of the renal tract, particularly vesicoureteral reflux, has long been recognized. Concern that episodes of pyelonephritis might result in significant damage to the kidneys culminating in chronic kidney disease (CKD) or even end-stage kidney disease has been tempered in recent times; moreover, any existing CKD is now recognized as predominantly congenital in origin. Recurrent infections, particularly febrile UTIs, do still however have accompanying morbidity. This chapter will address all aspects of childhood and adolescent UTI with particular emphasis on our current understanding of the pathophysiology, diagnosis, and management.

Keywords

Urinary tract infection · Pyelonephritis · *Escherichia coli* · *Diagnosis* · *Management* · *Antibiotics* · *Ultrasound* · Pediatrics · Cystitis · Immunity

Introduction and Background

In pediatrics, urinary tract infection (UTI) is the second most common bacterial infection affecting 7–8% of girls and up to 2% of boys in the first decade of life [1]. UTI is more predominant in males during the first year of life and girls thereafter and in most cases responds readily to treatment (Fig. 1). Among children who develop a UTI, up to 30% will develop a second infection. Over half of recurrent UTIs are caused by the same bacterial strain identified during the initial infection [2].

UTI is termed cystitis when the infection is confined to the bladder and urethra, with localized symptoms including dysuria, frequency, malodorous urine, and diurnal and secondary nocturnal enuresis. Acute pyelonephritis is a more significant infection that involves the renal parenchyma. Most affected children, particularly infants, appear systemically unwell with high fever, which may be the only feature. Febrile infections have caused the most concern as they place the kidney at risk of scarring with potential long-term sequelae. Asymptomatic bacteriuria is thought to be a variant of UTI by some and completely distinct from UTI by others, whereby a significant growth of organisms occurs on culture of the urine in the absence of any symptoms. It has been found on screening in approximately 1% of school-aged girls, but it does not require treatment or investigation as the outcome is excellent and uninfluenced by any intervention.

An association of UTI with kidney and urinary tract abnormalities has long been recognized, particularly when the infections are recurrent or febrile. When UTIs have been investigated with invasive imaging modalities, small hypo-dysplastic and scarred kidneys often

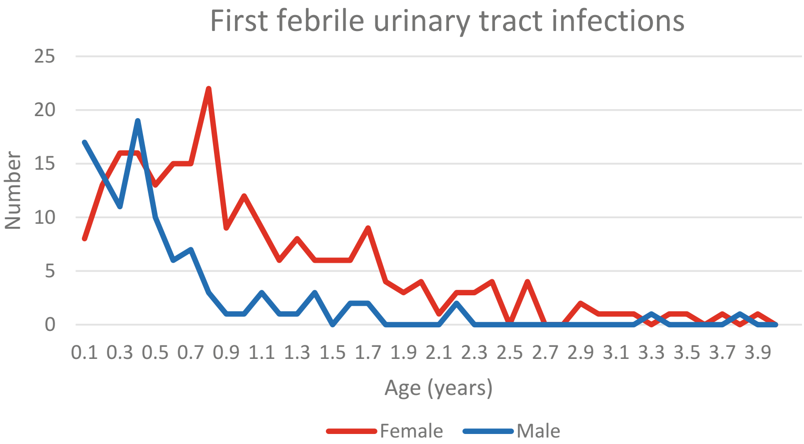

Fig. 1 Age at presentation of a first febrile UTI in 329 children (F 224, M 105) aged less than 4 years. Data abstracted from two prospective RCTs (IRIS 1&2)

with vesicoureteral reflux (VUR) were frequently found. The concept of reflux nephropathy was conceived to represent kidney damage predominantly attributed to ascending infections secondary to VUR resulting in renal scarring [3]. Children with reflux were often placed on long-term antibiotic prophylaxis or underwent ureteric reimplantation. Two early studies randomized children with VUR, detected following a UTI, to antibiotic prophylaxis or surgical correction alone or associated with adjuvant prophylaxis. Both studies demonstrated comparable satisfactory outcomes in the absence of a control (no treatment) or placebo group [4, 5]. The widespread introduction of prenatal ultrasound commencing in the 1980s demonstrated that much of the renal damage, often familial and detected in association with high-grade reflux, was the consequence of congenital abnormalities of the kidney and urinary tract (CAKUT) [6]. This led to a reevaluation of the role of acquired pyelonephritic scarring as a contributor to chronic kidney damage.

Clinical Presentation

There is considerable variability in the presentation of pediatric UTIs. UTI symptoms are influenced by the age at presentation, level of infection, nature and virulence of the organism, and the child's innate immune response. In newborns and infants, fever may be the only presenting symptom, while other nonspecific symptoms may include prolonged neonatal jaundice, irritability and listlessness, vomiting, slow feeding, and poor weight gain. There is a predisposition for UTI in infants to result in septicemia, including the possibility of meningitis [7, 8].

Symptoms localized to the urinary tract including frequency and dysuria are not usually apparent until 2–3 years of age. Flank pain secondary to pyelonephritis, even if present, is not generally reported by the child until 4–5 years of age. When the temperature is 38.5 °C or higher, the likelihood of renal parenchymal infection is increased, although cystitis per se can be present with fever.

In older children, voiding problems including hyperactive and dysfunctional bladder emptying may be associated with recurrent cystitis and less commonly recurrent febrile UTIs (see Chap. 59, Bladder Disorders). Thus, in these children a precise history of voiding patterns including continence, diurnal and nocturnal enuresis, frequency, urgency, and bowel habits is important [9, 10]. Although all children are susceptible to UTI, UTI risk is heightened in select populations (Table 1).

UTI Etiology

The common causative uropathogens originate in the fecal flora. *Escherichia coli* (*E. coli*) is the most frequent bacterial pathogen responsible for cystitis and pyelonephritis accounting for 85–90% of cases [2, 11]. However, other Gram-negative bacteria

Table 1 Pediatric populations with increased UTI susceptibility

Population	Rationale
Neonates and infants	• UTI risk has been attributed to an incompletely developed immune system.
Circumcision status	• In the first year of life, non-circumcised boys have increased UTI risk. • Uropathogens colonize areas under the prepuce, increasing the risk for urethral invasion and subsequent UTI .
Constipation and Gastrointestinal dysbiosis	• A stool-filled colon may compromise bladder emptying and increase UTI risk. • Antibiotic and immune-mediated disruptions of the gastrointestinal microbiota trigger inflammation and pathogen colonization that can increase UTI risk.
Immune response	• Alterations in the innate immune defenses may predispose children to UTI. • Polymorphisms in genes encoding pattern recognition receptors, cytokines, transcription factors, and host defense proteins are associated with childhood UTI predisposition .
Functional urinary tract disorders	• The inability to empty the bladder, with dysfunctional elimination syndrome or neurogenic bladder, results in urinary retention, urinary stasis, and inadequate pathogen clearance. • Elevated bladder pressures, secondary to poor drainage, may cause secondary VUR and potentiate kidney injury with pyelonephritis.
Anatomic urinary tract disorders	• Congenital kidney or urinary tract anomalies can result in urinary obstruction or stasis, increasing UTI susceptibility by reducing pathogen clearance. • Infections associated with urinary tract anomalies often appear in children younger than 5 years of age.
Systemic disorders	• Diabetes mellitus, sickle cell disease, and other immunocompromised states (HIV/AIDS, solid organ transplants) increase UTI risk.
Spinal dysraphism	• Myelomeningocele often leads to neurogenic bladder, which increases UTI risk. • People with spinal cord disorders may require intermittent catheterization which can lead to asymptomatic bacteriuria or UTI.
Sexual activity	• Sexual activity is a UTI risk factor in young women. • The proposed mechanism is the transfer of bacteria from the bowl or vagina to the urethral meatus during sexual intercourse.

colonizing the gastrointestinal tract cause UTI, including *Klebsiella, Pseudomonas, Proteus, Enterobacter, and Citrobacter* species. Certain Gram-positive organisms can also cause UTIs. *Enterococcus* species are the causative pathogens in both community- and hospital-acquired UTI. *Streptococcus agalactiae* is relatively common in newborn infants. *Staphylococcus saprophyticus* is seen in sexually active female adolescents, accounting for >15% of UTIs. In children with CAKUT or a compromised immune system, *Staphylococcus* and S*treptococcus* species also may be responsible [10, 11, 12]. Viruses and fungi are also relatively uncommon causes of UTI in children [13].

Cystitis Pathogenesis

Urinary tract infection occurs via the retrograde ascent of bacteria. Uropathogenic *E. coli* (UPEC) are excreted with the feces, colonize and spread across the perineum, ascend the urethra, and invade the bladder. Over the last two decades, a mosaic of UPEC virulence factors including adhesins, toxins, capsule composition, siderophores, and flagella have been identified to promote UTI (Fig. 2) [14]. To initiate cystitis, UPEC produce filamentous adhesive organelles called type 1 pili that bind uroplakins on superficial bladder urothelial cells. Adherence to the urothelium prevents UPEC from being cleared by the shear flow of urine during bladder emptying. Of note, atypical UTI pathogens that do not produce these adhesins are more likely to cause UTI when urine drainage is impaired (e.g., CAKUT, acute obstruction, nephrolithiasis, etc.) [15].

Attachment is the key event initiating UPEC UTI. Once bound, urothelial cells internalize UPEC. This internalization allows UPEC to enter a nutrient-rich cytosolic niche where they proliferate to form large, dense intracellular aggregates called intracellular bacterial

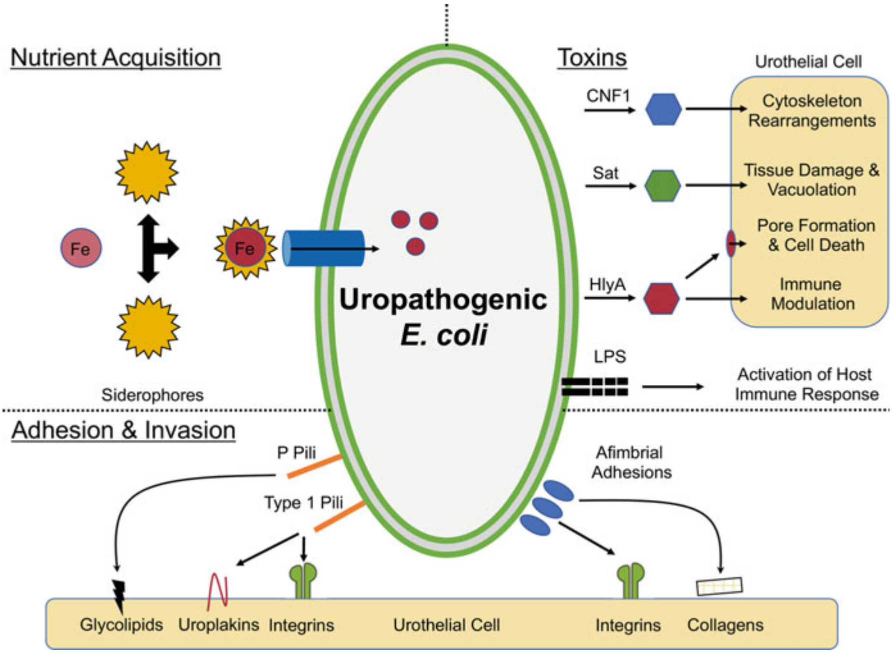
Nutrient Acquisition
Fe
Fe
Siderophores
Uropathogenic
E. coli
Toxins
CNF1
Sat
HlyA
LPS
Urothelial Cell
Cytoskeleton Rearrangements
Tissue Damage & Vacuolation
Pore Formation & Cell Death
Immune Modulation
Activation of Host Immune Response
Adhesion & Invasion
P Pili
Type 1 Pili
Afimbrial Adhesions
Glycolipids
Uroplakins
Integrins
Urothelial Cell
Integrins
Collagens

Fig. 2 Uropathogenic *E. coli*-associated fitness and virulence factors Shown is a representation of different factors that promote the virulence of uropathogenic *E. coli* (UPEC) strains. (**Left**) These factors include iron acquisition systems like siderophores. (**Right**) UPEC strains produce and secrete toxins that disrupt host membrane trafficking, cytoskeletal rearrangements, immune and inflammatory responses, and survival pathways. Depicted are the toxins that disrupt host cell integrity including cytotoxic necrotizing factor 1 (CNF1), secreted autotransporter toxin (Sat), and alpha-hemolysin (HlyA) as well as immune-activating proteins such as lipopolysaccharide (LPS). (**Bottom**) UPEC use multiple adhesins to bind proteins expressed on host bladder and kidney uroepithelial cells to cause infection [14]

communities (IBCs). IBCs develop from a single bacterium and ultimately contain up to 10^5 organisms that are encased in a highly organized polysaccharide matrix resembling an intracellular biofilm. IBCs are protected from antibiotics, neutrophil influx, and other host immune defenses. As the IBC matures, it engulfs the host nucleus [15, 16, 17, 18].

Within the IBC, UPEC replicate, differentiate, and develop filaments that extrude from the infected cell. Ultimately, UPEC dissociate from the urothelial cell and flux into the bladder lumen. Filamentous UPEC attach to neighboring bladder cells, reinvade the urothelium, and create additional IBCs. To limit infection, superficial urothelial cells containing IBCs are exfoliated to aid in the clearance of tissue-associated UPEC. This spurs the proliferation of intermediate and basal cells to regenerate and restore the urothelium [18, 19]. Exfoliated IBCs have been detected in the urine of women with UTI [20]. After exfoliation, UPEC may invade the underlying basal urothelium and establish a quiescent intracellular reservoir (QIR). Although the latent QIR population is small (containing only up to 12 bacteria), it is considered a major source of recurrent UTI [21, 22].

Pyelonephritis Pathogenesis

Although the pathogenesis of cystitis has been extensively investigated, the interactions between UPEC and kidney epithelial cells are less defined. Recent studies demonstrate that UPEC must be motile to establish pyelonephritis. UPEC strains lacking flagellin and the motor proteins MotA and MotB cannot ascend into the kidney [23, 24]. Upon reaching the kidney, UPEC concentrate in the medullary collecting tubules and preferentially attach to mitochondrial-rich α-intercalated cells. UPEC target intercalated cells because they are endocytically active and serve as an avenue of entry into the renal interstitium [25, 26, 27].

To bind medullary epithelial cells, UPEC must express type 1 fimbriae, which bind to the glycosphingolipids and mannosylated glycoproteins expressed on the surface of renal epithelial cells [28]. Translocation across kidney tubular cells occurs by two different mechanisms – paracellular and transcellular migration. UPEC possessing the virulence factor *hlyA* and *sat* (encoding α-hemolysin and a secreted vacuolating cytotoxin, respectively) activate caspase 3. Caspase 3 activation induces renal epithelial cell apoptosis, disrupting the tubular epithelial tight junction barrier and thereby promoting paracellular UPEC migration. UPEC strains lacking *hlyA* and *sat* cannot alter the tight junction complexes. Instead, these UPEC strains remodel the actin cytoskeleton and translocate across the cell in a Toll-like receptor 4 (TLR4)-facilitated fashion [26, 29].

Host Immune Response

The host utilizes several innate defenses to prevent UTI. Proposed antibacterial mechanisms limiting uropathogen attachment and invasion include epithelial barrier formation, the unidirectional flow of urine, routine bladder emptying, alterations in the urinary ionic composition, urinary and gastrointestinal microbiome homeostasis, and the production of antimicrobial peptides (AMPs) that inhibit bacterial attachment or directly lyse invading pathogens [30].

In the urinary tract, AMPs are produced by the uroepithelium of the bladder, ureter, and kidney tubules. In the kidney, intercalated cells are the primary AMP producers. The AMPs kill pathogens, disrupting the integrity of bacterial cell membranes, and have other antimicrobial assets. For example, AMPs block pathogen replication, bind specific targets to disrupt energetic processes, sequester vital nutrients like iron or other metals, promote bacterial opsonization, and interfere with bacterial toxins. In addition, AMPs interact with host cells to modulate innate and adaptive immune responses or promote epithelial regeneration. AMPs identified in the kidney and urinary tract include calprotectin, defensins, cathelicidin, hepcidin, lactoferrin and lipocalin 2, ribonucleases, and uromodulin. Cross-sectional studies in girls with recurrent UTIs suggest that deficient AMP production may be a UTI risk factor [15, 31, 32].

If bacteria overcome these innate immune defenses and attach to the bladder urothelium or kidney epithelium, they trigger a host inflammatory response that results in the production of distinct inflammatory mediators including inflammatory cytokines and chemokines, complement activation, and AMPs [31, 32, 33]. The bladder urothelium and kidney's intercalated cells play key roles in orchestrating these effectors. This response is followed by the activation of innate immune cells and proteins that migrate to the infectious focus and facilitate eradication of the invading bacteria. While these innate immune responses effectively eradicate invading pathogens, the resulting inflammation may lead to tissue injury [15, 32].

Mechanisms of Kidney Scarring

Although it is well recognized that acute pyelonephritis (APN) in children may lead to kidney scarring, the pathogenesis or mechanisms by which kidney inflammation leads to tubulointerstitial fibrosis are not well defined. Evidence indicates that kidney scarring is a consequence of excessive inflammation and bacterial infiltration [34, 35]. This inflammatory reaction, which is triggered to eradicate invading bacteria, leads to tubular cell injury, macrophage and neutrophil infiltration, and activation of pericytes and myofibroblasts. Within the kidney, the activated myofibroblasts are widely accepted to be the primary cellular driver of fibrosis. Myofibroblast activation is driven by transforming growth factor beta, which is secreted by injured tubules and activated macrophages [36]. In addition to myofibroblast activation, experimental evidence shows that dysregulated macrophage-dependent inflammatory responses promote kidney damage and fibrosis [37].

Unlike other kidney injury paradigms such as unilateral ureteral obstruction or ischemia-reperfusion injury, APN leads to an ascending pattern of fibrosis [38, 39]. With APN, fibrosis initiates deep to the urothelium in the kidney's pelvis and is identified by expansive submucosal connective tissue deposition. This collagenous deposition spreads through the interstitium to the outer medulla and cortex. Ultimately, this gives rise to the wedge-shaped patterns of renal parenchymal injury identified in children following APN [40, 41].

Diagnosis of Urinary Tract Infection

UTIs are diagnosed upon the culture of a single organism in sufficient numbers from an appropriately collected urine specimen. Laboratories employ a standardized technique sampling 0.001–0.002 ml (1–2 μL) of urine in a sterile loop plating it out on a culture medium and incubating in room air at 35° C, with the plates checked for the type and number of colonies. Dipslide cultures provide an alternative, particularly for after-hours collection of specimens, thus enabling earlier reporting of culture results. Urinalysis is a useful adjunct in the diagnosis, particularly as culture results may take 24–48 hours, but never obviates the need for urine culture. Various methods of urine collection for culture are employed including suprapubic bladder aspirate (SPA), catheter specimens (CSU), midstream-voided specimens (MSU), and bag specimens each method having progressive increases in the likelihood of bacterial contamination. The threshold level for a significant growth of organisms to define a positive culture based on an MSU was first set by Kass 60 years ago at $\geq 10^5$ organisms/ml, based on pyelonephritis studies in pregnant women [42]. Kass acknowledged at the time that such a threshold would result in missed UTIs, which is also true in the pediatric population. A recent study of infants <1 year of age with a symptomatic UTI diagnosed by suprapubic aspirate demonstrated that 19% had colony counts $<10^5$ of whom 87% had counts $<5 \times 10^4$ [43]. An editorial comment on this subject noted that diagnosing a UTI based on a strict threshold level was inappropriate [44]. Most published guidelines on diagnosis of UTI in children avoid a detailed discussion of what constitutes an infection. Recently the Diagnosis of Urinary Tract infection in Young children (DUTY) study attempted to validate a clinical algorithm for the diagnosis of UTI in those who presented with an acute illness in the primary care setting. Pads were used for sample collection in infants, and clean-catch specimens were used in older children. Unfortunately, disagreements persisted on UTI diagnosis between government and research facilities, although most agreed that clean catch was the preferred collection method [45].

Contamination during collection proved a particular problem creating difficulty with interpretation of urine cultures. Contamination can occur during collection by organisms from the urethra or periurethral area, genitalia, and surrounding skin. Suprapubic aspiration of urine and samples obtained by percutaneous nephrostomy is considered the "gold" standard for diagnosis of UTI, due to the reduced risk of contamination, with any organism cultured generally considered to be significant. Ideally, suprapubic aspiration is performed with ultrasound guidance; however, the invasive nature (leading to patient and family objections) and skill required for the procedure often preclude its use. Urethral catheterization is the next most effective method of obtaining a culture specimen with a growth of 10^3–10^4 colonies per ml typically considered significant. As with suprapubic aspiration, parents may consider this stressful for the child, with the additional concern that infective organisms may be introduced by the procedure. A midstream-voided specimen represents the most practical method, particularly after children are toilet trained and able to void on request. Presently, most agree that a positive culture consists of one with growth of a single organism $\geq 10^5$ colonies per ml or $\geq$ 50,000 colonies per ml of a single organism accompanied by pyuria [46]. Many groups (and some national guidelines) believe that midstream samples should have both a significant colony count on urine culture and urinary leukocytes for the diagnosis of UTI [47]. The presence of pyuria can assist in the differentiation between contamination, colonization, and clinically significant infection. When sterile leukocyturia is present, urine antibacterial activity should be sought out; if no antibiotics were administered prior to urine collection for culture, consideration should be given to the presence of atypical bacteria such as *Ureaplasma* and/or *Gardnerella* species that require non-standard culture media. In addition, other inflammatory conditions such as hypercalciuria or Kawasaki disease should be considered along with tuberculosis in endemic areas. The culture of multiple organisms increases the likelihood of contamination such that a culture should be repeated [9].

While not always successful, clean-catch urine specimens may be possible in infants and young children as they void frequently. One study reported a success rate of 86% in newborns, using a bladder and lumbar stimulation technique to obtain a midstream urine specimen with a median time of sample collection of 43 seconds [48]. Bag collections are practiced in primary care settings [49] and are of use as a screening procedure to perform urinalysis. If leukocyturia is present, a definitive urine collection and culture such as an MSU or CSU should be performed. The use of bag collections as the sole method to perform a culture is discouraged and contraindicated in all published guidelines [50].

A presumptive diagnosis of UTI and subsequent treatment is often guided by the findings on preliminary urinalysis and clinical presentation, prior to the culture results. If a UTI is strongly suspected, such as when leukocyturia and fever are present, it is appropriate to initiate antibiotic treatment before culture results, taking into consideration local drug sensitivities and resistance patterns. Urine samples can be examined by microscopy and/or urine dipsticks. Urine dipsticks are quick, easy to perform, and cost-effective. As noted above, leukocyte esterase positivity in the urine is a strong indicator of pyuria. As regards the nitrite test, most uropathogens produce nitrite from nitrate, although it may take up to 4 h to generate sufficient nitrite. This explains why infants and children with urinary frequency often have a false-negative test. Gram-positive infections, such as those from *Enterococcus*, will also not present with nitrites. A positive nitrite test cannot be relied upon in isolation, as it can be positive with asymptomatic bacteriuria and hence requires significant pyuria for conformation [47]. Recently the revised Italian guidelines note that the presence of white blood cells in the urine, with or without a positive nitrite test, allows for the presumptive diagnosis of UTI [47]. Hence, urine dipsticks offer a fast, easy, and cost-effective means of directing the initial management and treatment. Future studies are needed to determine the most suitable method of urine sampling, the correct thresholds for the culture counts depending on the methods of urine collection in children, and the significance of positive urine cultures in the absence of leukocyturia.

Differentiating Upper from Lower Urinary Tract Infections

The differentiation between upper and lower tract UTIs is not always apparent during the acute presentation. No markers exist that reliably predict the presence of acute pyelonephritis. C-reactive protein and white cell counts are often elevated but are insufficiently sensitive and specific for the diagnosis of renal parenchymal infection. Procalcitonin, a calcitonin precursor, released by the thyroid gland in response to bacterial infections, is currently the most reliable blood test of pyelonephritis and of subsequent renal scarring [51]. A number of urinary markers have been shown to increase during pyelonephritis as opposed to cystitis. These include INF gamma, IL15, and chemokine ligands CXCL1, CXCL9, and CXCL12; however none has proved better than procalcitonin [52]. The “gold standard” for the diagnosis of acute pyelonephritis remains the technetium-99 m-labeled dimercaptosuccinic acid (DMSA) scan (see Chap. 6 on Diagnostic Imaging). In the past, this imaging test had been proposed as an initial investigation within the first week of infection. Presently its use has declined in this context, given the significant radiation dose and lack of availability in many non-tertiary centers. Potential blood and urine markers are continuing to be evaluated by investigators. For practical purposes, fever in association with a UTI is considered pyelonephritis in the clinical setting [53, 54].

Treatment of an Acute UTI

With their advent, antibiotics have become the cornerstone for UTI treatment resulting in dramatically improved outcomes. In the early twentieth century, infants and young children hospitalized with acute pyelonephritis frequently died as a consequence of their disease [55]. Despite the recognized effectiveness of antibiotics in treating UTIs, until the mid-1990s little research had been conducted on the appropriate agent(s), manner of administration, and duration of treatment. Uncomplicated afebrile UTIs were and generally continue to be treated with oral antibiotics with a high renal excretion and a more narrow spectrum. A recent systematic review of antibiotic treatment for uncomplicated lower UTI in children concluded that short-course oral therapy (3–7 days) was as effective as long-course (7–10 days) treatment; however, single-dose therapy is not recommended until further studies are undertaken to determine its effectiveness [56].

In contrast, treatment for febrile UTIs, particularly in infants and younger children, typically included hospital admission and broad-spectrum intravenous antibiotics. Between 1995 and 2004, five clinical trials demonstrated that short courses of intravenous antibiotics (up to 4 days) followed by oral therapy were as effective as longer courses of intravenous treatment (7–14 days) [57, 58, 59, 60, 61]. Four studies involving a total of 1344 infants and children as young as 1 month of age with a first febrile UTI went further, demonstrating that oral administration of antibiotics was as effective as initial intravenous followed by oral antibiotics. The studies showed no difference in time to resolution of fever or in occurrence of subsequent renal damage [62, 63, 64, 65].

Most current guidelines for treatment of a febrile UTI recommend oral antibiotic administration on an outpatient basis, with hospital admission and parenteral administration restricted to those children who appear to be severely ill, who are vomiting and/or with whom poor adherence is a risk [66, 67]. The choice of antibiotic should be guided by local antibiotic resistance patterns and aimed toward the most common causative pathogenic bacteria. Amoxicillin/clavulanate and cephalosporins are typically used antibiotics both for oral and iv administration, where local sensitivities permit. In many areas of the world, UPEC producing extended spectrum beta-lactamase (ESBL) is of concern [68]. Ampicillin-sulbactam could be used as first-line treatment, depending on local availability [47]. Antibiotics commonly used and dosages suggested by different guidelines are listed in Table 2. Provided an organism is sensitive, no antibiotic has been shown as superior to another. Many support the use of iv cephalosporins (cefotaxime or ceftriaxone) in children with severe infections, as these antibiotics have a superior efficacy and onset of action, making the possibility of resistance a less likely. In the case of a breakthrough infection in children on antibiotic prophylaxis, a change of antibiotic becomes necessary, because of an almost certain resistance to the prophylactic agent [69].

Infants in the first month of life who present with unstable temperature, lethargy, poor feeding, and/or prolonged jaundice may be septic and warrant intravenous antibiotics after blood and urine cultures are taken. Sepsis in the first 72 hours is most commonly from *group B streptococcus*, although UPEC infections can occur in this period and become more common thereafter. Neonatal sepsis is recognized as a serious illness, particularly when it occurs in low birth weight preterm infants. Bacterial infections in infants tend to be more severe due to an immature immune system and, in the case of preterm infants, reduced placental transfer of protective maternal antibodies. Thus, intravenous penicillin class antibiotics combined with aminoglycosides are commonly used on an empiric basis. Care should be taken to monitor aminoglycoside levels because of their potential nephrotoxicity, particularly in infants with impaired renal function secondary to renal dysplasia.

Table 2 Antibiotics and dosages recommended by international guidelines for the treatment of UTI

	AAP	CARI	ISPN	Comment
Oral antibiotics mg/kg/day				Oral antibiotics preferred in well-appearing children as equally efficacious to iv therapy
Amoxicillin-clavulanic acid	40 in 3 doses	22.5 + 3.2 in 2 doses	50–90 in 3 doses	
Cephalexin	50–100 in 4 doses	50 in 4 doses	–	
Cefixime	8 (max: 400/day) in 1 or 2 doses	–	8 twice the first day once daily thereafter	
Ciprofloxacin	Not recommended as empiric treatment	25 in 2 doses*	20–40 in 2 doses*	*use where resistance to other abs or *Pseudomonas* infection
Trimethoprim-sulfamethoxazole	6–12 (trimethoprim) in 2 doses	8 (trimethoprim) in 2 doses	8–12 (trimethoprim) in 2 doses	
Intravenous antibiotics mg/kg/day				Recommended in children who appear septic or cannot tolerate oral abs. Change to oral when clinical improvement
Ampicillin-sulbactam	100–200 (ampicillin) In 4 doses		100 (ampicillin) in 3–4 doses	
Amoxicillin-clavulanic acid			100 (amoxicillin) in 3–4 doses	
Cefotaxime	150–180 in 3 doses	75 in 3 doses	150–200 in 3–4 doses	
Ceftriaxone	75 single dose	25 single dose	75–100 single dose	
Amikacin	15–22.5 single dose		15 single dose	Need to adjust dosage for renal function and serum levels
Gentamicin	5–7.5 single dose	<10 years: 7.5 ≥ 10 years: 6 single dose	6–7.5 single dose	

Local drug recommendations should always be consulted regarding contraindications, side effects, maximum prescribed dosage, and duration of therapy. The NICE (National Institute for Health and Care Excellence) guidelines do not provide specific details on drug dosages. *AAP* American Academy of Pediatrics, *CARI* Caring for Australian and New Zealanders with Kidney Impairment, *ISPN* Italian Society of Pediatric Nephrology

Imaging

No clear consensus exists regarding imaging after a UTI, although a better understanding of infections and their sequelae has led to fewer procedures. Most existing protocols focus on imaging in infants and younger children with febrile UTIs; however, few recommendations exist regarding afebrile UTIs and/or those occurring in older children. Some of the imaging modalities that can be considered in these patients are listed below (also see Chap. 6 on Diagnostic Imaging).

Ultrasonography

Ultrasonography can detect a variety of anatomical abnormalities and is noninvasive but is dependent upon the expertise of the sonographer. The widespread implementation of antenatal ultrasound has been of value in detecting the majority of significant congenital abnormalities of the kidney and urinary tract (CAKUT), including kidney hypodysplasia, obstruction at the pelviureteric and vesicoureteric junctions, and posterior urethral valves [70]. Kidney and bladder ultrasound (KBUS), performed after a febrile UTI, did not reliably reveal the presence of VUR or subsequent scarring in three prospective trials, involving 864 children. Minor congenital abnormalities that had little influence on subsequent outcomes were found in 12 to 14% of cases [71, 72, 73].

The value of performing a routine KBUS following an uncomplicated febrile UTI has been increasingly questioned (Table 3). The American Academy of Pediatrics (AAP) [46] and the Italian Society of Pediatric Nephrology (ISPN) [66] recommend US in all children under 2 years of age following an initial febrile UTI; however, the National Institute for Health and Care Excellence (NICE) guidelines restrict US to <6 months of age [67], and the Australian guidelines (CARI) do not recommend US except in the absence of an antenatal study or in the presence of other risk factors [74].

Given the paucity of KBUS findings following an uncomplicated UTI in childhood in areas where second and/or third trimester antenatal US shows normal anatomy, most believe a KBUS is unnecessary. However, in areas where access to antenatal care and health services is limited, most recommend that a KBUS be performed. If the infection is atypical (caused by a non-UPEC organism, lacks an appropriate antibiotic response), renal damage is suspected, a weak urine stream occurs, or recurrent infections are present, then a KBUS should be performed [75, 76, 77]. In older children, a KBUS is suggested after recurrent UTIs, once a detailed voiding history has been assessed. In these patients, the KBUS should also be used to evaluate bladder filling and emptying, the residual urine post micturition, and bladder wall thickness both full and empty (the bladder wall full is usually less than 3 mm and empty less than 5 mm) (see Bladder Disorders, Chap. 59). Significant abnormalities on bladder ultrasound warrant consideration of urodynamic studies.

Voiding Cystourethrography

Voiding cystourethrography (VCUG) involves serial imaging of the low urinary tract following the instillation via urethral catheterization of a radiopaque contrast media. VCUG tests can detect the presence of VUR, urethral obstruction due to posterior urethral valves in boys, ureteroceles, and/or bladder changes such as those consistent with a neurogenic bladder. This study has led to controversy given the distress this invasive procedure can cause parents and children. Advocates of VCUG, predominantly surgeons [78], cite a strong association between the severity of reflux and presence of renal damage [75], now recognized as predominantly congenital rather than acquired [79, 80].

Most guidelines support a selective approach to this investigation, citing the lack of evidence that medical or surgical interventions are typically useful, except possibly in children with high-grade reflux [81]. Most recommend a VCUG in children following a febrile UTI in the first 2–3 years of life if the KBUS is abnormal or the UTI atypical (Table 3). The Italian guidelines also identify specific US abnormalities that represent an indication to perform a VCUG, including uni- or bilateral renal hypoplasia, abnormal renal parenchymal echogenicity, ureteral dilatation, uroepithelial thickening of the renal pelvis, and pelvi-calyceal dilatation, particularly if associated with bladder uroepithelial thickening, and other bladder abnormalities [82]. Regarding atypical UTI, a recent article has confirmed that the presence of a pathogen other than UPEC significantly predicts reflux [83]. Thus, a reasonable approach would include a VCUG following a first febrile UTI in children less than 2 years of age with major abnormalities of the urinary tract or kidneys on KBUS and a poor urine stream or when a non-UPEC infection is diagnosed. Recurrent febrile UTI also represents an indication for VCUG. In older children with febrile UTIs or recurrent cystitis, most believe that a VCUG is rarely indicated, since an ultrasound and detailed history of voiding patterns are usually sufficient to establish the diagnosis and initiate treatment.

Table 3 Published guidelines on the investigation and management of a first febrile UTI

Guideline	Ultrasound	VCUG	DMSA	Prophylaxis
NICE (2007, updated 2017)	Atypical, < 6/12 age	No unless <6 months of age with positive US or atypical UTI	Yes >6/12 post UTI	No
AAP (2011)	Yes	No unless abnormal US	No	No
ISPN (2020)	Yes	No unless abnormal US or bacteria other than UPEC	Consider if major abnormalities at US	Considered for VUR IV-V
CARI (2014)	Yes, if: No second or third trimester US, <3/12 age, or atypical UTI	No unless abnormal US	No	No
Canadian (2014)	Yes	No unless abnormal US	No	No

NICE National Institute for Health and Care Excellence, *AAP* American Academy of Pediatrics, *CARI* Caring for Australian and New Zealanders with Kidney Impairment, *ISPN* Italian Society of Pediatric Nephrology, *VCUG* voiding cystourethrography

Renal Scintigraphy

Renal imaging with a radionucleotide dimercaptosuccinic acid (DMSA) scan is useful in assessing renal morphology and function, producing static images. The test entails the intravenous administration of a radioactive isotope that localizes to the renal parenchyma. Decreased uptake of isotope as seen on a gamma camera several hours postinjection can confirm pyelonephritis if performed within 1 week following a febrile UTI or determine if scarring has resulted if undertaken 6–12 months later. Delaying the test to diagnose a renal scar is important as uptake defects from pyelonephritis can persist for several months. The DMSA scan can also differentiate congenital renal hypodysplasia from acquired scarring that may otherwise be difficult to discern. The former typically appears as a smaller kidney with uniform uptake of isotope, while the latter usually manifests as a focal uptake defect with disruption of the normally smooth kidney contour.

The AAP guidelines make no recommendation regarding an "early" or "late" DMSA scan following a febrile UTI [46], NICE guidelines recommended a late DMSA scan when the infection is atypical [67], and the ISPN does where high-grade VUR is present (Table 3) [47]. An alternative promoted by some is the "top-down" approach whereby a DMSA scan is performed as an initial investigation in the acute phase of the illness, followed by VCUG if the scan is positive. Two studies reported a strong correlation between abnormal DMSA scans and high-grade VUR. In contrast a more recent study demonstrated a normal DMSA scan during the acute infection in 14/46 children with grade III–IV reflux [84]. A late DMSA scan has been recommended by some, in order to detect those children with renal scarring that require follow-up [73, 85]. DMSA scans are not indicated in patients that have afebrile UTIs.

Two other isotopes that are useful for renal nuclear medicine imaging include technetium-99 m-labeled diethylenetriaminepentaacetic acid (DTPA) and technetium-99 m-mercaptoacetyltriglycine (MAG3). Both have a dynamic early excretory phase that is useful for determining the existence and severity of obstruction, when dilatation of the urinary tract is noted on a structural imaging study such as ultrasound. ^{9m}TC-MAG3 renal scintigraphy has also been used in indirect cystography but has low sensitivity in detecting VUR of any grade and cannot, therefore, be proposed as an alternative to VCUG in the diagnosis of VUR (or for posterior urethral valves) [86].

Other Imaging Modalities

Intravenous pyelography has been virtually completely replaced by KBUS and radionuclide scintigraphy. Magnetic resonance urography (MRU) allows for the precise evaluation of anatomical features and less so functional imaging while avoiding radiation exposure. MRU has limited capacity to evaluate obstruction, i.e., measuring cortical transit times of contrast media. Currently no guidelines recommend MRU due to the need to sedate the child. With further development of the technology, MRU may become more cost-effective and a viable alternative to currently used imaging modalities [87].

Antibiotic Prophylaxis

Long-term low-dose prophylactic antibiotics administered following an uncomplicated first febrile UTI have been shown in prospective studies to be of minimal or no value in the prevention of breakthrough UTIs or subsequent risk of scarring. In the past, antibiotic prophylaxis had been extensively prescribed following pyelonephritis, mainly in the presence of VUR, on the unproven hypothesis that it reduces UTIs and avoids kidney scarring [88]. Eight prospective randomized controlled trials (2390 children) evaluated the effectiveness of prophylaxis [89, 90, 91, 92, 93, 94, 95, 96]. Given the disparate populations, duration of follow-up, and investigations performed, it is difficult to compare the studies directly; however, some conclusions are possible. Recurrent UTI was the primary endpoint in all papers. Antibiotic prophylaxis showed no benefit compared to no treatment in four studies evaluating children with absent or mainly low-grade VUR [89, 90, 91, 92]. The Swedish Reflux Trial, conducted in 203 infants with grade III–IV reflux, reported a reduction in febrile UTIs on prophylaxis compared with controls (19% vs 57%, $p < 0.001$) over a 2-year period in girls, but not boys [94]. The two largest placebo-controlled studies, the PRIVENT (576 children, 64% female, 61% absent or grade I–II VUR) and RIVUR (607 children, 92% female, 92% grade I–III VUR) trials, enrolled 1183 children with a significant reduction in breakthrough UTIs in the antibiotic prophylaxis arm. This result is statistically significant, although some believe less clinically relevant, given the need of 14–16 patient years of prophylaxis to prevent one symptomatic UTI and 22 patient years of treatment to prevent one febrile UTI [93, 95]. The benefit of prophylaxis was seen predominantly in the initial 6 months, in both papers. The RIVUR study showed that the largest benefit for reduction of subsequent UTI with prophylaxis was in patients with bowel and bladder dysfunction. Overall, a systematic review of these two studies demonstrated a slight benefit of prophylaxis in reduction of recurrent UTI, at the same time as an increase in bacterial resistance. The severity of VUR and the appearance of scarring were not addressed [97].

The risk of renal scarring was evaluated with a late DMSA scan in seven of the previous studies. Only the Swedish Reflux Trial showed a reduction of scarring in female infants with high-grade VUR, on prophylaxis [81]. Importantly, no single study had adequate power to demonstrate a benefit of prophylaxis in regard to renal scarring. Furthermore, a systematic review and meta-analysis of 1427 subjects from the previous seven RCTs was unable to show that antibiotic prophylaxis prevented renal scarring, as did a sub-analysis restricted to the 1004 subjects with vesicoureteral reflux [98]. A shortcoming of these trials relates to the populations enrolled, as many had absent or low-grade reflux and the majority were female (92% in the RIVUR study) and these patients are expected to have a good outcome regardless of intervention. The population at risk for progressive kidney disease, males with high-grade VUR, often in association with renal (hypo)dysplasia and bladder issues, were underrepresented and frequently excluded by enrolment criteria.

Various guidelines no longer support routine antibiotic prophylaxis in children with VUR (Table 3). In children with recurrent febrile UTIs and grade IV–V reflux, for whom prophylaxis might be beneficial, there is little agreement regarding the duration of the prophylaxis. One approach is to consider 2 years in females and 1 year in male infants who have recurrent febrile UTIs and high-grade reflux. Nitrofurantoin is an appropriate first-line agent, although side effects and risk of poor adherence often preclude its use. Cotrimoxazole or amoxicillin/clavulanate are often preferred.

Antibiotic Prophylaxis and Risk of UTIs in Different Urological Disorders

Major urological malformations particularly those involving the bladder commonly predispose to UTI. Persistent bladder dysfunction can be seen with a range of disorders such as posterior urethral valves, neurogenic bladder, and bladder exstrophy, each often managed with clean intermittent catheterization, and as such pose risks for bacterial colonization of the bladder and recurrent UTIs. These children are no longer treated with antibiotic prophylaxis; the one randomized controlled trial on clean intermittent catheterization in children demonstrated a significantly higher incidence of UTIs in the prophylaxis arm compared with those with no treatment [99]. In addition, a meta-analysis examining the use of antibiotic prophylaxis in older children and adults with spinal cord dysfunction did not support prophylaxis [100]. Even with these data, there is considerable disagreement in how to best manage patients with persistent bladder dysfunction.

One report showed that avirulent commensal organisms frequently colonize neurogenic bladders in children [101]. These organisms did not appear to cause symptomatic UTI or decline in kidney function, despite prolonged colonization. Thus, asymptomatic bacteriuria in this population does not warrant treatment.

The role of antibiotic prophylaxis in infants and young children with high-grade VUR and chronic renal insufficiency due to (hypo)dysplasia, who are at high risk of deteriorating renal function, has not been evaluated in a prospective controlled trial. A particularly vulnerable population are newborns, who have not experienced a UTI, but that were diagnosed with congenital renal abnormalities on prenatal US. An international multicenter randomized controlled trial (PREDICT trial) to evaluate prophylaxis in the above population has completed enrollment, with the final results expected at the end of 2021 (ClinicalTrials.gov Identifier: NCT02021006).

Additional Strategies to Prevent UTI

Circumcision

Circumcision for the prevention of UTIs has not been evaluated in prospective randomized trials [102]. Two systematic reviews of manly cohort studies reached conflicting outcomes. In the first, routine circumcision was not recommended in normal boys, as the number needed to treat was calculated as 111 to prevent 1 UTI. The authors suggested that it could be an option when recurrent UTIs or high-grade reflux are present [103]. In the second, circumcision was recommended in all boys, as it reduced the lifetime risk of UTI, with approximately four circumcisions to prevent one UTI [104].

Cranberry

Consumption of cranberries (or cranberry juice) has been reported to inhibit the adhesion of UPEC to the endothelial cells of the urinary tract and thus be of possible benefit in reducing UTIs. A Cochrane review of 24 trials, however, did not show a benefit of cranberry consumption in the prevention of recurrent UTI. The trials were undertaken in a variety of clinical settings, including in patients with neuropathic bladders and/or spinal injury [105].

Probiotics

Probiotics have been proposed as a possible means of reducing recurrent UTIs. Most studies have been performed in women rather than children using different strains of organisms (predominantly lactobacilli), and results are conflicting [106]. One study showed a slight benefit in prevention of UTIs using probiotic prophylaxis over a 6-month period [107]. The paper had methodologic weaknesses that preclude widespread recommendations regarding probiotic use.

Constipation

Constipation has long been recognized as a risk factor for recurrent UTIs. One study of 234 chronic children with constipation, wetting issues, and infections demonstrated that successful treatment of the constipation resulted in cessation of diurnal enuresis in 89%, nocturnal enuresis in 63%, and UTI in all [108]. The treatment of constipation and soiling in "at-risk" children is clearly important in the prevention of recurrent UTIs (see Bladder Disorders, Chap. 59).

Surgical Correction of Vesicoureteral Reflux

Surgical correction of reflux, a common procedure in the past, is no longer routinely recommended. The surgery may be considered in patients with breakthrough febrile UTIs despite prophylaxis, particularly in association with high-grade reflux in females (see Vesicoureteral Reflux, Chap. 59).

Future Clinical UTI Sequelae

Most uncomplicated UTIs have limited long-term outcomes. Febrile UTIs have a high likelihood of inflammation at the renal parenchymal level, leading to possible renal scarring, which may be responsible for long-term adverse events. Without scarring, long-term issues are typically absent. Overall, renal scarring following a febrile UTI has been reported with a prevalence of 15% in a systematic review [109] and appears unrelated to age [110]. A meta-analysis of 9 studies involving 1280 children and adolescents showed that scars developed in 191 children; those with the highest rates included those with fever >39 °C and ultrasound abnormalities, in whom the causative organism was not UPEC [111]. Conflicting views exist regarding the relation between the magnitude of scarring and potential long-term adverse outcomes.

Widespread use of antenatal ultrasound over the past several years has shown that renal parenchymal damage previously attributed to pyelonephritis is usually the consequence of congenital maldevelopment of the kidneys. This maldevelopment is frequently accompanied by anomalies of the lower urinary tract, including high-grade VUR and/or obstruction. Long-term sequelae of concern include hypertension, pre-eclampsia, and chronic kidney disease. A comprehensive review highlighted the finding that older studies, predominantly retrospective from biased populations, recorded a high incidence of adverse outcomes [112]. More recent publications that are population based and/or that exclude congenital renal disease by prenatal ultrasound report a much lower incidence of harmful sequelae. Only 0.4% of 1029 children recruited in prospective trials with normal renal function at the outset reported significant declines on follow-up. The vast majority of children with impaired renal function at the end of follow-up had had scarring or renal dysplasia at the time of enrollment. The same review evaluated 713 children from 5 studies and found that the incidence of hypertension increased from 2.4% to 4.6% during 5 to 20 years of follow-up [113, 114, 115, 116, 117]. Those studies showing a high incidence of hypertension, scarring, and renal damage in patients at follow-up were in those patients where these findings were present at study onset. One study with a follow-up of 16–26 years, evaluating 53 children with scars and 51 without after a first UTI, was unable to show any difference in blood pressure profiles by ambulatory blood pressure monitoring between the two groups [118].

Regarding pregnancy complications, 1 study evaluated 72 women (median age of 41) who had given birth and who had a prior history of childhood UTI and found an overall low complication rate. Hypertension was diagnosed in 10 out of 151 pregnancies and preeclampsia in 4, all in women with renal damage. All women were normotensive before their first pregnancy [119]. While this prospective study is limited, it does provide reassuring long-term data on effects of childhood UTI on later complications.

Conclusions

UTI is a common problem in pediatric and adolescent patients. One of the clinical challenges of diagnosing a UTI includes possible vague presenting symptoms, especially in young children. Thus, a high index of suspicion is appropriate when a young child presents for evaluation with fever without other explanation. Although the gold standard to diagnose a UTI is a urine culture, clinicians routinely rely upon their clinical suspicion and the results from a urine dipstick test to decide on whether to initiate therapy. Oral antibiotic therapy for febrile UTI in young children should last 7–14 days and be modified if necessary based on the urine culture results. The appropriate work-up after a UTI in infants, children with an atypical UTI, or lack of prenatal imaging often includes a KBUS. Children with renal scarring after a UTI should be followed long term for possible hypertension and/or chronic kidney disease.

Cross-References

- ▶ Bladder Disorders
- ▶ Imaging Kidneys and the Urinary Tract
- ▶ Infections That Affect the Kidney (Nonviral)
- ▶ Pediatric Obstructive Uropathy
- ▶ Vesicoureteral Reflux in Children

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Vesicoureteral Reflux in Children

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Abstract

Vesicoureteral reflux (VUR) is the most common congenital anomaly of the urinary tract in children. Primary VUR is diagnosed mostly after a urinary tract infection (UTI). It is also diagnosed during postnatal evaluation of antenatally diagnosed hydronephrosis or other congenital anomalies of the kidney and urinary tract (CAKUT). High-grade VUR ($\geq$ grade 3) is a risk factor for acute pyelonephritis-associated acquired reflux nephropathy. Other important risk factors for reflux nephropathy include recurrent UTIs and a delay in initiation of antibiotic treatment. Not all children with a first febrile UTI need a voiding cystourethrogram (VCUG), but it should be considered in those with abnormal ultrasound findings, recurrent UTIs, and/or an atypical causative pathogen. The management of VUR depends on multiple factors and may include surveillance only or antimicrobial prophylaxis; very few patients with VUR need surgical correction. Patients with reflux nephropathy should be monitored for hypertension and those with significant bilateral scarring should be monitored for proteinuria and chronic kidney disease.

Keywords

Vesicoureteral reflux (VUR) · Urinary tract infection (UTI) · CAKUT · Bladder-bowel dysfunction (BBD) · Renal scarring · Reflux nephropathy · Antimicrobial prophylaxis · VCUG · DMSA

Synonyms

Reflux; Urine back-flow

Introduction

Vesicoureteral reflux (VUR) may be primary or secondary. Primary VUR is the most common congenital anomaly of the urinary tract and is usually diagnosed after UTI or during follow-up of antenatally diagnosed hydronephrosis (see ▶ Chap. 55, "Urinary Tract Infections in Children"). In contrast, secondary VUR typically develops after a surgical procedure such as a renal transplant, decompression of a ureterocele, correction of vesicoureteral junction obstruction, or due to increased bladder pressure. Increased bladder pressure may occur due to anatomical obstruction caused by posterior urethral valves or urethral or meatal stenosis (see ▶ Chap. 57, "Pediatric Obstructive Uropathy") or a functional bladder abnormality as seen with neurogenic bladder, nonneurogenic neurogenic bladder (Hinman's syndrome), and Ochoa syndrome (see ▶ Chap. 58, "Bladder Disorders"). Transient VUR may occur in patients with bladder bowel dysfunction or bladder infection. In this chapter, we will discuss primary VUR because it is the most common type diagnosed in otherwise healthy children.

Incidence and Prevalence

The exact incidence of primary VUR is not known because it is not feasible to do a VCUG in a large cohort of healthy children. Its reported prevalence varies from 1.3% of healthy children to anywhere from 8% to 50% of children evaluated after UTI [1]. In newborns and infants, the incidence of VUR diagnosed after UTI is 36–49% [1]. Children with VUR detected after UTI are predominantly females [2, 3]. VUR diagnosed during follow-up of antenatally diagnosed hydronephrosis is seen more often in males [4]. VUR is less common in African American children and typically less severe as compared to Caucasian children [5]. Eighty-one percent of the 607 patients in the Randomized Intervention in Children with VUR (RIVUR) study were Caucasian [6].

Embryology and Anatomy

The ureterovesical junction is designed to prevent backflow of urine from bladder to the kidney. The ureters pass obliquely into the bladder through the detrusor. The distal end of the ureter is located submucosally within the bladder and when the submucosal tunnel is short, this can predispose to VUR. The muscle fibers of the ureter extend into the trigone of the bladder and are interwoven with the fibers from the opposite ureter. The intermingling of fibers allows fixation of the ureters within the trigone of the bladder. While no true valve exists at the ureter-bladder junction, the distal submucosal ureteral segment compresses against the muscular bladder wall during bladder filling, occluding the ureteral orifice and preventing the reflux of urine. Finally, the tone of the ureter and the anatomical location of the ureteral orifice within the trigone also prevent reflux.

The ureteric bud is an embryonic primordial structure that gives rise to the final metanephric kidney and the ureter (see ▶ Chap. 1, "Pre-natal Development of the Kidneys and Urinary Tract"). The precise location of where the ureteric bud emerges is critical to the formation of the kidney and the ureter since both tissues require the correct milieu of transcription factors and signaling molecules for their development. If the ureteric bud emerges from a more caudal location along the mesonephric duct, the kidney that forms may lack the proper complement of nephrons and/or even exhibit signs of dysplasia [7]. In addition, the final location of the ureteral orifice may not be within the trigone, but in a more lateral and proximal location. The abnormally located orifice arises with a shortened submucosal ureteral tunnel that is predisposed to VUR. Indeed, the shared embryonic origin of the kidneys and ureters is one explanation for the co-occurrence of VUR and renal dysplasia or congenital reflux nephropathy that is typically observed in boys (see ▶ Chap. 3, "Congenital Anomalies of the Kidneys").

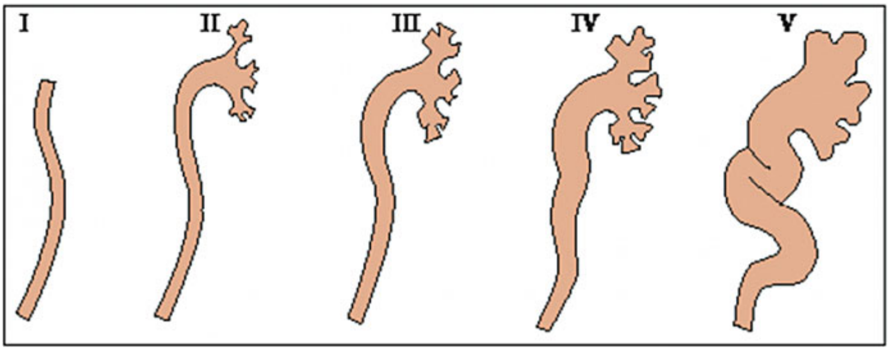

Fig. 1 International grading **system for vesicoureteral reflux** (International Reflux Study grading scheme. Grade I – reflux into non-dilated ureter. Grade II – reflux into the renal pelvis and calyces without dilatation. Grade III – mild/moderate dilatation of ureter and pelvicalyceal system. Grade IV – dilation of the renal pelvis and calyces with moderate ureteral tortuosity, blunting of fornicies. Grade V – gross dilatation of the ureter, pelvis, and calyces; ureteral tortuosity; loss of papillary impressions)

Grading of VUR

The gold standard for diagnosing VUR is a voiding cystourethrogram (VCUG). VUR is classified into 5 grades (Fig. 1). Grade I is reflux into the ureter, grade II is reflux into the renal pelvis without any dilation of the calyces, grade III is reflux to the renal pelvis with mild dilation of the renal pelvis, grade IV is reflux to the renal pelvis with greater dilation of the renal pelvis, and grade V is reflux to the renal pelvis with ureteral and pelvic dilation. The RIVUR study revealed that there is a considerable inter-observer variability in the VUR grading by a VCUG. Radiologists varied by one grade in about 20% of patients and rarely by two or more grades [8].

Radionuclide voiding cystogram has also been used to diagnose and follow children with VUR. However, using this modality, reflux can only be graded into mild, moderate, and severe as the resolution is not as good as with a contrast VCUG.

Natural History of VUR

Primary VUR generally improves with time, and this is attributed to the lengthening of the submucosal segment of the ureter with overall growth of the body. Other factors associated with resolution of VUR include low grade at diagnosis, nonwhite race, and the absence of bladder bowel dysfunction [9]. In one study, the mean time until spontaneous resolution in black children was 14.6 months, which was significantly shorter than the mean of 21.4 months in white children [10]. Unilateral VUR has a better probability of resolution as compared to bilateral VUR. One study in 735 children with primary VUR examined the median time for persistence of reflux over a follow-up period between 6 and 411 months; the authors found median resolution times of 38 months for grade I/II, 98 months for grade III, and 156 months for grade IV/V VUR [11]. The rate of resolution of VUR may be higher in younger children. In a 5 year follow-up study in children less than 5 years old, grade I VUR resolved in 82%, grade II in 80%, and grade III in 46% of the ureters [12]. The rate of resolution is better in nondilated as compared to dilated ureters. Schwab and colleagues reported that grades I-III VUR resolve at a rate of 13% per year for the first 5 years of follow-up and 3.5% per year during subsequent years; moreover, grades IV and V VUR resolve at a rate of 5% per year [13].

Clinical Presentation of Primary VUR

Primary VUR is often diagnosed in one of three ways – following UTI, during follow-up for congenital abnormalities of the kidney and urinary tract (CAKUT)/antenatal hydronephrosis, or while screening a sibling of a patient with VUR.

UTI and VUR

UTI, particularly in younger children, is the most common presentation that leads to the diagnosis of VUR. In a large cohort of children with UTI, VUR was diagnosed in 46% of infants and 9% of preschool children [14]. The majority of children with VUR diagnosed after a UTI are girls, although when considering infants <6 months of age, no gender difference is noted [15].

CAKUT/Antenatal Hydronephrosis and VUR

VUR is the most common abnormality observed in children with CAKUT [1]. In children with prenatal hydronephrosis, postnatal evaluation with VCUG reveals the presence of VUR in 10–30% of infants [16]. However, as is the case with UTI, the number of VCUG tests requested for this indication has also been decreasing over time. The incidence of VUR is higher in boys with antenatal hydronephrosis [4]. VUR associated with antenatal hydronephrosis has a high rate of spontaneous resolution (59% by age 4 years) even in those with grades IV–V reflux [17]. Uncircumcised male infants with prenatal hydronephrosis and VUR have a significantly higher incidence of UTI even in those prescribed antibiotic prophylaxis [18].

Siblings and VUR

A study showed that 66% of children that had a parent with VUR also had VUR indicating a significant parent to child transmission [19]. Studies by Jerkins and Noe revealed that 32% of siblings of index children with VUR also had VUR [20]. Review of the natural history of VUR in siblings indicated that 52% had resolution of reflux after 18 months of follow-up, with yearly resolution rates of 28% [21]. A more "benign" course of "sibling reflux" compared to reflux identified after a UTI [22] has led many to question the usefulness of screening of asymptomatic siblings. Currently, no routine screening for siblings of a diagnosed child with VUR is advised unless the sibling develops a UTI.

Bladder Bowel Dysfunction

VUR may be associated with bladder bowel dysfunction (BBD) which is also called voiding dysfunction or dysfunctional elimination syndrome (see ▶ Chap. 58, "Bladder Disorders"). The exact pathogenesis of BBD is not known. It appears to occur from difficulties with toilet training, evolving from poorly learned voiding technique in which the child attempts to suppress impending or active bladder contractions by inappropriately contracting the pelvic floor muscles and tightening the external sphincter [23]. This results in increased bladder pressure and/or inefficient bladder emptying [24]. Bladder dysfunction is invariably associated with constipation. BBD can be assessed using a questionnaire called the dysfunctional voiding scoring system [25]. The symptoms of BBD include daytime wetness, urgency, frequency, infrequency, recurrent UTI, dysuria, abdominal pain, constipation, and/or fecal incontinence in "toilet trained" children with no underlying anatomic or neurological abnormality.

In a study of 366 children with symptoms of BBD, 77% of the patients were females and the mean age at referral was 8.5 years (range, 4–18 years). Constipation/encopresis was reported in 30% cases, day-time wetting in 89%, night-wetting in 79%, and recurrent UTI in 60% of the patients. VUR was present in 20% of the patients who underwent cystourethrography [26]. In the RIVUR study, using the dysfunctional voiding scoring system [25], BBD was identified in 56% of 126 toilet-trained children (<6 years of age) with grade I to IV VUR [6].

BBD predisposes to recurrent UTI, induces and perpetuates VUR, and may result in permanent renal damage. BBD increases the risk of febrile UTI in children with VUR on antimicrobial prophylaxis (44% with BBD and 3% without

BBD) and it delays VUR resolution at 24 months (31% with BBD and 61% without BBD) [27]. The frequency of postoperative UTI is greater in children with BBD (22%) compared to those without BBD (5%) [27]. BBD can also adversely affect the results of ureteric reimplantation [28]. The frequency of UTI after open or endoscopic surgical correction of VUR is greater in children with BBD (22%) compared to those without BBD (5%) [27]. In summary, BBD is a relative contraindication for surgical correction of VUR because of a higher likelihood of treatment failure and the potential for recurrent UTI [27].

BBD is a diagnosis of exclusion that mandates a detailed history and physical examination to rule out any underlying neurological or neuromuscular etiology. Urinalysis, bladder ultrasound, and VCUG in selected cases are helpful in making a diagnosis. The role of full urodynamic studies with catherization is not well-established, in part because study results are not consistent. The procedure is invasive and the results may not change therapy or influence the final outcome. However, evaluation of the bladder with a postvoid residual and assessment of the urine flow can be useful in the evaluation of BBD related to VUR. Multidisciplinary management of BBD with dedicated pediatric nurses and a psychologist facilitated the resolution of VUR in 53% of patients after a mean follow-up period of 22 months [26].

Constipation is an invariable part of BBD, but may be an isolated finding, which alone increases the risk of recurrent UTI in children with VUR. Constipation causes compression of the bladder and the bladder neck secondary to stool in the colon. The fecal load increases the bladder storage pressure and the residual urine volume postvoid. Also, the distended colon and/or soiling provide an abundant reservoir of pathogens, which may affect the profile of the stool microbiome and increase the diversity of species [29–31]. Constipation in children increases the likelihood of urinary incontinence, bladder overactivity, dyscoordinated voiding, recurrent UTI, and persistence of VUR [31].

VUR and Renal Scarring

In 1960, Hodson and Edwards described the co-occurrence of VUR with chronic pyelonephritis [32], which subsequently came to be known as reflux nephropathy (RN) [33]. Pyelonephritis-induced renal injury in the presence of VUR is called acquired RN. This differentiates it from congenital RN, which is a result of abnormal renal development and not secondary to injury from pyelonephritis. Congenital RN is more common in boys, whereas acquired RN due to febrile UTI is more common in girls [34]. Some other important differences between acquired and congenital RN are shown in Table 1 [35]. In previous studies on reflux nephropathy, inclusion of patients with preexisting renal injury without clinical pyelonephritis may have falsely increased the number of patients reported to have acquired RN. In more recent randomized studies, renal scarring correlated with gender, with males demonstrating more renal scarring. This could be attributed to the higher incidence of congenital RN observed in boys [36].

The gold standard for diagnosing RN is dimercaptosuccinic acid scintigraphy (^{99m}Tc-DMSA). The DMSA renal scan does not differentiate between congenital and acquired RN, which may look similar on imaging. The DMSA

Table 1 Congenital versus acquired reflux nephropathy in children (Modified from Mattoo TK [35])

	Acquired reflux nephropathy	Congenital reflux nephropathy
Time of occurrence	Postnatal	Prenatal
History of UTI before diagnosis	Common	Uncommon
Age distribution	All pediatric age groups	Mostly in younger children
Gender distribution	Predominately females	Predominately males
Grade of VUR at the time of diagnosis	Variable depending on age	Mostly high grade
Renal histology	Chronic inflammatory changes	Features of renal dysplasia

UTI: Urinary tract infection, VUR: Vesicoureteral reflux scan should be performed at least 4–6 months post-UTI. This allows the acute inflammatory reaction associated with pyelonephritis to subside, at which point any persistent cortical defects can be assumed to represent permanent renal scarring. However, in the absence of a baseline (pre-acute pyelonephritis) scan, it may be difficult to differentiate acquired from congenital lesions.

The reported prevalence of renal scarring after febrile UTI is about 15% [37]. Scarring ranges from 3% after the first UTI to 29% after more than three febrile UTIs [38, 39]. Renal scarring occurs with acute pyelonephritis with or without VUR, but the risk is higher in the presence of VUR. The role of VUR in renal scarring has been reported in multiple clinical studies and has been shown to be causally linked to scarring in a pig model [40].

Mechanism of Renal Injury

Several studies have shown that scarring develops at the same sites where bacteria infect the renal parenchyma. Renal scarring due to intrarenal reflux, that is, when urine refluxes from the renal pelvis into the calyces, occurs mostly at the renal poles. Morphologic evaluation demonstrates that extensively fused (compound) papillae are associated with intrarenal reflux and occur almost exclusively at the upper and lower poles of the kidney [41]. Renal scarring may result from a single episode of pyelonephritis, especially in young children, also known as the "big bang effect" [42] or the process may take several years. After acute pyelonephritis, renal scarring can take months to be detected on a DMSA renal scan [43].

The exact pathogenesis of renal scarring following acute pyelonephritis is not well understood, but from animal models, it is clear that inflammation is central to the development of a renal scar. The innate immune response of the urinary tract refers to immediate host responses which clear the bacterial infection. The innate immune response includes recognition of bacteria via specific pathogen recognition receptors located on uroepithelial cells, the Toll-like receptors (TLRs), recruitment of neutrophils, and production of proinflammatory cytokines like interleukin (IL)-8 and IL-6 [44, 45] (see ▶ Chap. 55, "Urinary Tract Infections in Children"). Uroepithelial cells express TLR4 which senses lipopolysaccharides in the cell wall of Gram-negative bacteria like *E. coli* and activates a signaling cascade resulting in the release of IL-8. This, in turn, recruits neutrophils and other inflammatory cells from the bloodstream into the urinary tract [46, 47]. This rapid neutrophil response is essential for clearing a urinary tract infection and is largely driven by IL-8 signaling through its receptor, IL-8R.

According to Roberts and colleagues, the acute inflammatory response that eradicates the invading bacteria also appears responsible for early renal parenchymal damage and subsequent scarring [48]. The early release of IL-6 and IL-8 is a chemoattractant for leukocytes that migrate to the site of infection where they exert a microbicidal effect through phagocytosis. During phagocytosis, neutrophils and monocytes release lysosomal enzymes and superoxides, to ingest the invading bacteria. The inflammatory response is also accompanied by tubular ischemia and reperfusion injury. Antioxidant-mediated renal tubular injury is attributed to the release of superoxides during tissue reperfusion [49, 50]. The fibrosis that follows is initiated mainly by activated fibroblasts or myofibroblasts that secrete collagen. Myofibroblasts may possibly arise from differentiation of tubular epithelial cells to fibroblasts, epithelial-mesenchymal transition (EMT), from endothelial to fibroblast differentiation as part of endothelial-mesenchymal transition, from proliferation of local fibroblasts and pericytes or as part of a macrophage to myofibroblast transition [51]. Cytokines produced as part of inflammation include transforming growth factor-beta 1, platelet-derived growth factor, and fibroblast growth factor, which attract and stimulate the proliferation of fibroblasts that are ultimately responsible for collagen production and scarring. Explanations for progressive parenchymal renal injury even after VUR has ceased include hyperfiltration of intact nephrons, the presence of Tamm-Horsfall protein in the interstitium as a putative antigenic determinant, superoxide production, and persistent hypertension. Hyperfiltration of intact nephrons causes glomerulosclerosis, activation of the renin-angiotensin system, and a gradual deterioration of renal function [52, 53].

Risk Factors for Renal Scarring

A number of risk factors are associated with acquired renal scarring. These include recurrent UTI, high grade VUR, duration of fever of > 72 h prior to antibiotic initiation, and organisms other than *E. coli*. There is also evidence that genetic risk factors predispose children with VUR to renal scarring.

Recurrent UTI

Recurrent UTI is a well-known risk factor for renal scarring [36, 54]. In the RIVUR study, children with a second UTI had significantly more renal scars [OR], 2.85; 95% confidence interval [95% CI], 1.38 to 5.92) as compared to those with a single UTI [36]. A post hoc analysis of RIVUR and CUTIE [Careful Urinary Tract Infection Evaluation] studies revealed that the odds of renal scarring after a second febrile UTI was 11.8 (95% CI, 4.1–34.4) times greater than after a single febrile infection and the odds of renal scarring after three or more febrile infections was 13.7 (95% CI, 3.4–54.4) times greater than after a single febrile infection [55].

Vesicoureteral Reflux

High-grade VUR, grade III or higher, is an important risk factor for renal scarring. Children with high-grade VUR are more likely to develop scarring than children with lower grades (RR: 2.1 [95% CI: 1.4–3.2]) [56]. The odds ratio of renal scarring with acute pyelonephritis in the presence of VUR versus no VUR is 2.8 for patients and 3.7 for renal units [27]. Another systematic review reported that children with VUR have a greater likelihood of developing acute pyelonephritis and renal scarring than children without VUR [56]. A meta-analysis of the published data revealed that renal damage (per 100 renal units) occurred in 6.2% in those with grades I–III VUR and 47.9% in those with grades IV and V VUR [57]. In the RIVUR study, the proportion of new scars in renal units with grade 4 VUR was significantly higher than in units with no VUR (OR, 24.2; 95% CI, 6.4 to 91.2) [36].

Delay in Treatment

Delay in the treatment of febrile UTI increases the risk of renal injury. A multivariate analysis of 158 children with febrile UTI showed that a delay of greater than 48 h in the initiation of antibiotic treatment was associated with an increased risk of acute lesions on renal scan [58]. The RIVUR study revealed that delayed initiation of antibiotic treatment was associated with an increased risk of scarring, with the odds ratio of new renal scarring 74% lower among children whose treatment started within 24 h of onset of fever, compared to those whose treatment started after 72 h of fever [55]. Young children with pyelonephritis also have higher rates of other complications such as sepsis and abscess formation when treatment is delayed.

Patient Age

Recent studies have shown that young age may not be a risk factor and that older children may be at a higher risk of renal scarring [36–38]. In the past, young age was thought to be a risk factor likely due to the inadvertent inclusion of patients with preexisting congenital scarring (renal dysplasia). Renal scarring occurs in adult kidneys as well, as is seen in adult transplanted kidneys [59]. Also, adult pig kidneys with VUR and UTI scar as quickly/easily as those of piglets [60].

Complications of Renal Scarring

The complications of renal scarring are well known, but poorly defined because of their insidious onset, slow progression, and the lack of well-designed prospective studies in children as well as in adults. Hypertension and proteinuria are the most common complications. Other complications include focal segmental glomerulosclerosis (FSGS), urinary concentration defects, hyperkalemia, and acidosis [61, 62]. In most children, renal scarring may not be clinically significant [63].

Proteinuria

Renal parenchymal injury may cause persistent proteinuria, which may lead to nephrotic syndrome in some cases. Overt proteinuria, which has been reported in 21% of adult patients with RN [64], is rare in pediatric patients. The pathogenesis of proteinuria in RN is not well understood and is attributed to immunologic injury, macromolecular trapping and mesangial dysfunction, vascular alterations and hypertension, and glomerular hyperfiltration. Of these explanations, the one that seems most plausible is hyperfiltration in remnant nephrons. This results in modifications in selective permeability to macromolecules such as albumin and progression of renal disease [65, 66]. The histologic hallmark in such patients is hypertrophy of surviving nephrons with focal and segmental glomerulosclerosis [67]. FSGS is progressive and can occur in nonscarred parts of the kidney or in the normal contralateral kidney in patients with unilateral RN [61].

Microalbuminuria, which indicates glomerular damage at a very early stage, precedes the development of overt proteinuria, progressive renal damage, and renal failure. Microalbuminuria has been reported in 51% of pediatric patients (mean age 9.8 + 4.2 years) with renal scarring [68] and increases with the severity of renal scarring [69, 70].

Hypertension

Hypertension occurs in 10–30% of children and young adults with renal scarring [71, 72] and 34–38% of adult patients with renal scarring [64, 73]. In adults, confounding factors such as renal scarring due to renal stones and other renal pathologies make interpretation difficult. In a study involving 664 patients diagnosed with VUR between 1970 and 2004, 3% of patients developed hypertension. The estimated probability of hypertension was 2%, 6%, and 15% at 10, 15, and 21 years of age, respectively. The survival analysis revealed that about 50% of children with unilateral and bilateral renal damage develop persistent hypertension at about 30 and 22 years of age, respectively [74]. In a follow-up period lasting 15 years in pediatric patients with renal scarring, about 13% of patients at age 20–31 years were hypertensive [75].

The exact cause for hypertension due to renal scarring is not known, but is believed to be due to segmental ischemia with increased renin secretion. It does not depend on the severity of renal scarring. In a 5-year prospective study of patients with longstanding reflux nephropathy, there was no correlation between blood pressure (BP) and plasma renin activity (PRA), plasma creatinine concentration, or the degree of scarring [76].

Some studies have reported no increase in BP after many years of follow-up. In a study of 146 subjects with VUR diagnosed at a mean age of 5 years, no hypertension was seen after a mean follow-up of about 10 years. The cohort included 34.3% patients with renal scarring [77]. In another study of 53 patients with renal scarring following childhood UTI, there was no significant difference in ambulatory BP measurements (ABPM) 16–26 years later, compared to 47 matched subjects without scarring [63]. However, in a follow-up study in 86 adult women (median age 41 years) with UTI in childhood, 24-h ABPM was used to diagnose hypertension. The mean 24-h systolic, mean daytime systolic, and mean night-time SBP were significantly higher in women with renal damage compared to those without renal damage [78].

Chronic Kidney Disease (CKD)

RN is the 4th most common cause of renal failure in children with CKD, in children on dialysis, and in children who have received a renal transplant according to the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) reports [79]. In the CKID study of 586 children aged 1–16 years, with an eGFR of 30 to 90 mL/min/1.73m^2, RN was the underlying cause of CKD in 87 (14.8%) patients [80].

In a study that evaluated the clinical course of 735 children with primary VUR between 1970 and 2004, the probability of CKD was reported as 5% at 10 years after diagnosis of VUR; for children diagnosed after 1990, the probability of CKD was only 2%. This suggests that the prognosis for patients diagnosed with VUR may be improving over time [11]. Another study by the same group evaluated independent predictive factors of CKD in children with severe bilateral grades III-V VUR. The authors found that the probability of CKD for patients with bilateral severe reflux was 15% by 10 years after VUR diagnosis. The three variables that were independently associated with CKD were age at diagnosis >24 months [relative risk (RR) = 4.8, 95% confidence interval (95%CI), 1.8–12.7, $P<0.001$], VUR grade V (RR = 3.5, 95%CI, 1.5–7.9, P = 0.002), and bilateral renal damage (RR = 2.86, 95%CI, 1.3–6.1, P = 0.007). In contrast, admission after 1990 was a protective factor (RR = 0.16, 95%CI 0.06–0.43, $P<0.001$). The study concluded that the long-term renal prognosis in patients with bilateral severe reflux is poor [81]. The long-term follow-up of patients with RN who have undergone renal transplantation appears to be good. The Australia and New Zealand Dialysis Registry reported on first kidney transplants performed between 1985 and 2015 in individuals less than 30 years of age with a diagnosis of CAKUT. The 10-year graft survival in individuals with CAKUT was favorable: patients with RN had a 70% graft survival at 10 years and 49% at 20 years [82].

Complications in Adults with VUR Diagnosed in Childhood

The complications of renal scarring are more common in adults, who may present with recurrent UTI, proteinuria, hypertension, and increased serum creatinine. Adult males with RN generally present with hypertension, proteinuria, and renal failure (Table 2). Females present mostly with UTI and pregnancy-related complications [35]. Males have poorer outcomes compared to females. This difference has previously been attributed to a delayed diagnosis in males because of an insidious onset of proteinuria and renal failure. In contrast, the diagnosis may be discovered earlier in females due to recurrent UTI and pregnancy-related complications. However, it is also possible that gender differences in adults may be due to the fact that males are more likely to have congenital RN with renal dysplasia which may lead to greater renal damage than acquired RN, which is seen more in females [35].

In a study of 293 adults with RN (mean age at presentation 31 + 13.4 years), females outnumbered males by a ratio of 5:1. The most common presenting complaints for both genders were UTI (65%), hypertension (20%), and proteinuria (6%). However, UTI as a presenting complaint was significantly more common in females compared to males (72% vs. 31%). In contrast, proteinuria was significantly more common in males (20% vs. 4%). Hypertension was also more common in males than females (29%

Table 2 Reflux nephropathy in adults [35]

	Males	Females
Urinary tract infections	Uncommon	Common
Plasma creatinine	Higher	Normal
End-stage renal failure	More common	Less common
Vesicoureteral reflux	High grade/bilateral	Low grade/unilateral
Proteinuria	Frequent/severe	Infrequent/Less severe
Hypertension	More common	Less common

vs.18%). Although VUR typically resolves over time, VUR was present in 65% of the females and 75% of the males when tested as adults: this suggests the cohort had severe high grade VUR. Of the 147 patients who were followed for a mean period of 6.9 + 3.6 years, 21 (14%) developed ESRD after a mean follow-up of 7.0 + 3.1 years [83]. In a study of 21 adults (mean age 23.9 years) with gross VUR diagnosed in infancy, proteinuria and renal insufficiency was diagnosed in three (23%) of the 13 patients with unilateral reflux nephropathy and two (50%) of the four patients with bilateral reflux nephropathy [84]. In another study of 127 adults (mean age 41 years) with VUR diagnosed during childhood, 44 (35%) had unilateral renal scarring, 30 (24%) had bilateral renal scarring, 30 (24%) had albuminuria, and 14 (11%) had hypertension. Of the 30 patients with bilateral renal scars, 25 (83%) had an abnormal GFR [85].

The potential complications during pregnancy in women with RN include UTI, hypertension, proteinuria, edema, deterioration in renal function, preterm delivery, preeclampsia, and fetal loss [86]. The degree of renal function at conception is associated with the outcome during pregnancy. A review of the literature on the outcome of pregnancy in women with a history of VUR revealed that VUR increases the risk of UTI from 6% to 22%, which increases to 42% when associated with renal scarring. The incidence of hypertension and preeclampsia was significantly higher in women with VUR and renal scarring compared to women with VUR without scarring [86].

Congenital or acquired RN may account for a significant number of adults who commence renal replacement therapy. CAKUT including CAKUT related to VUR leads to end stage kidney disease more often in adulthood than in childhood. In a study of approximately 200,000 patients over 20 years, 2.2% of the patients commenced renal replacement therapy due to a diagnosis of CAKUT. In fact, the median age at renal replacement therapy initiation was 31 years in the CAKUT cohort versus 61 years in the non-CAKUT cohort. CAKUT was diagnosed using ERA-EDTA primary disease codes. The group of patients with VUR and pyelonephritis represented the largest fraction of the CAKUT diagnoses, accounting for 41.9% of the group. Their mean age to commence renal replacement therapy was 38.5 years, indicating that they are developing end stage kidney failure as relatively young adults [87].

Genetics of VUR, UTI, and Renal Scarring

Genetic studies of VUR have been informative to understand the pathogenesis of this disorder and have revealed mutations in genes that are causal for VUR. Some of the limitations of the genetic studies relate to small sample sizes, the clinical variability of study subjects, difficulties in diagnosing asymptomatic carriers especially since VUR may resolve with age, and the impact of environmental factors that may augment phenotypic variability.

From animal models, it is evident that specific gene defects lead to a refluxing uretero-vesical junction. There are also genetic risk factors that predispose to recurrent urinary tract infections and to an impaired host immune response that promotes renal scarring [88, 89]. The first suggestion that primary VUR could be a genetic disorder came from case reports in which monozygotic twins were found to have VUR. Additional support included the fact that VUR frequently affects multiple family members, siblings of children with VUR are more likely to be affected [20, 90], and the fact that there are differences in the incidence of VUR when comparing different ethnic groups [5]. The strongest evidence demonstrating that primary VUR is heritable is a study of 46 twin pairs in which 80% (12/15) of the monozygotic twins had concordant VUR versus 30% (11/31) of the dizygotic twins [91]. When only the youngest affected twin pairs were analyzed, monozygotic twins showed an even higher concordance for VUR at 100% (7/7) versus 50% of the dizygotic twins (5/10). Monozygotic twins are genetically identical and give an estimate of the effect of shared genes, while dizygotic twins give an estimate of the effect of the shared environment. If a disorder shows higher concordance among monozygotic twins versus dizygotic twins, this is strong evidence that a disorder is genetically transmitted.

All genetic inheritance patterns have been reported in primary VUR including monogenic dominant [92, 93], monogenic recessive [94, 95], and complex inheritance when genetic risk factors interact with environmental conditions to predispose to a disorder [96]. A number of genetic approaches have been used to identify genes and loci that are relevant for VUR. This includes genetic analysis of rare patients with VUR and defined chromosomal aberrations. This approach was utilized to identify the *ROBO2* gene as a candidate gene when it was discovered that an individual with a chromosomal translocation that disrupted *ROBO2* also had multiple congenital anomalies including severe bilateral VUR [97]. SLIT2 is a ligand produced by the mesonephric duct. It signals to its receptor ROBO2 that is expressed by the adjacent nephrogenic mesenchyme and together this signaling initiates formation of the ureteric bud [98] (see ▶ Chap. 1, "Prenatal Development of the Kidneys and Urinary Tract"). A number of candidate genes have been sequenced in cohorts of patients with VUR based on their putative roles during kidney/urinary tract development. Some are implicated in the formation of a refluxing uretero-vesical junction, while in other cohorts, the mutations in these same genes have not been identified. Some of these candidate genes include *RET*, a receptor tyrosine kinase expressed by the ureteric bud [99] and *PAX2* a critical transcription factor for kidney and urinary tract development. *PAX2* is associated with a rare syndrome known as renal-coloboma syndrome that also encompasses VUR [100]. Other candidates include the angiotensin type 2 receptor, *AGTR2* [101], and the uroplakin genes, *UPK2* and *UPK3* that are expressed in the uroepithelium of the urinary tract and bladder and affect the morphology of the uretero-vesical junction [102, 103].

Other nonbiased genetic approaches have focused on whole genome-wide mapping of informative families to identify loci and the underlying causal genes. These studies have identified loci on almost every chromosome [95, 104, 105]. Replication studies to validate these loci have generally not been successful, which suggests that primary VUR is genetically heterogeneous. In the largest study to date of 460 families in Europe, parametric linkage analysis using a dominant model revealed linkage to a region on chromosome 10q26 encompassing 69 genes [106]. Gbadegesin et al. using a similar approach performed sequential-wide linkage on a large 97 member family with VUR and joint hypermobility and mapped the disorder to a locus on chromosome 6p [107]. Using whole exome sequencing, they identified a causal heterozygous missense variant in *Tenascin XB*, *TNXB*, a large extracellular matrix protein that regulates collagen deposition. This nonbiased genetic approach to discovery brings a new hypothesis to the understanding of VUR and suggests that perturbations in the extracellular matrix that supports the uretero-vesical junction may predispose to VUR. These observations are particularly compelling since children with VUR frequently demonstrate joint hypermobility [108, 109].

Perturbations in the host immune response can predispose to pyelonephritis and to renal fibrosis. In mice, mutations in *Tlr4* are associated with low-grade chronic pyelonephritis and/or an asymptomatic carrier state known as asymptomatic bacteriuria from failure to initiate the signaling cascade required for neutrophil influx [110–112]. Coding mutations in *TLR4* have not been identified in humans with urinary tract infections, but sequence variation in the *TLR4* promoter is associated with a decrease in TLR4 expression and asymptomatic bacteriuria [113, 114]. Mice bearing a mutation in the IL-8 receptor, *mCxcr2*, can recruit neutrophils to the renal interstitium but the neutrophils are unable to efficiently migrate across the uroepithelium into the urinary tract, leading to acute pyelonephritis and renal fibrosis [115–117]. The findings in the mouse correlate with a human study describing reduced expression of the IL-8 receptor, CXCR1, in a cohort of acute pyelonephritis-prone children [118]. These studies are critical to understanding why some patients with VUR and pyelonephritis never develop reflux nephropathy, while others do. A few candidate genes involved in the host immune response and renal fibrosis have been examined in patients with reflux nephropathy and in patients with acute pyelonephritis. For example, a polymorphism in the Nod-like receptor, *NLRP3*, which senses bacterial pathogens to activate the innate immune response, is associated with acute pyelonephritis in children. A recent study examined copy number variants in children from the RIVUR cohort and found that copy number variants (CNVs) implicated in the innate immune response were enriched in children with VUR compared to age and ethnicity-matched controls [119]. The study of genetic risk factors for UTIs and renal scarring remains an important avenue for future research, which ultimately may lead to new treatments to treat and/or prevent renal scarring.

Diagnosis of VUR and Renal Injury

Ultrasonography

Ultrasonography is the initial modality for the evaluation of postnatal hydronephrosis and UTI in children (see ▶ Chap. 6, "Imaging Kidneys and the Urinary Tract"). It is not a very sensitive imaging modality for the diagnosis of VUR. For example, in the RIVUR study, only 11% of the patients had an abnormal US examination as defined by the presence of hydronephrosis or ureteral duplication [6].

Use of the renal resistive index from color Doppler ultrasound has also been used to follow affected children with VUR, but it has not received widespread acceptance due to its poor correlation with VUR [120]. Color flow Doppler sonography has been used to identify retrograde ureteral jets at the uretero-vesical junction as a sign of VUR. Good correlation was noted with the VCUG [121]; however, the detection of ureteral jets was not sensitive enough to diagnose VUR in all cases. In addition, the child is required to urinate in a supine position, which may be difficult.

Renal US also has very low sensitivity for diagnosing acute pyelonephritis. In one study, US abnormalities compatible with acute pyelonephritis were reported in 20–69% of patients with acute pyelonephritis as compared to 40–92% by DMSA scintigraphy [122]. Nonetheless, a renal ultrasound is useful to diagnose congenital anomalies of kidney and urinary tract and to detect complications of acute pyelonephritis such as renal abscess or pyonephrosis.

Renal ultrasound is also not a sensitive method for diagnosing renal scars. In a study of 159 patients who underwent DMSA renal scan as well as renal US for the evaluation of hypertension of unknown etiology, thirty-three (21%) patients with normal ultrasound examination were found to have renal scars by DMSA. The sensitivity and specificity of renal US, to diagnose renal scars was 36% and 94%, respectively [123].

Voiding Cystourethrogram

VCUG is the primary diagnostic modality for identifying and grading VUR (see ▶ Chap. 6, "Imaging Kidneys and the Urinary Tract"). Concerns that a VCUG performed soon after a UTI would unmask low grades of insignificant reflux have not been borne out by studies to date [124]. The VCUG should be obtained as soon as the child has completed the course of antibiotic therapy for UTI. It should be done selectively in patients with abnormal ultrasound examination, recurrent UTI, UTI with an atypical pathogen, or known renal scarring.

The results of the VCUG can be affected by the size, the type, and the position of the catheter, the rate of bladder filling, the height of the column of contrast media, the state of hydration of the patient, and the volume, temperature, and concentration of the contrast medium. Utilization of sedation to perform the VCUG may compromise the results as children may be unable to void to completion when sedated. The presence and grade of VUR is also affected by the number of times that the bladder is filled during the VCUG. In a study that involved multiple fillings and emptying of the bladder, Jequier reported a discrepancy in the presence and/or grade of VUR from one filling to the other in 12% of patients with two cycles and in 20% of patients with three cycles. No changes were observed with multiple fillings and emptying in cases of grade IV reflux [125]. The aforementioned inter-observer variability in the grading of VUR must be kept in mind when making clinical decisions [8].

The number of VCUG tests done in children on antibiotic prophylaxis for VUR has decreased steadily because of concerns with radiation exposure. However, the current use of low-dose radiographic equipment, including digital fluoroscopy, has reduced radiation exposure [126, 127]. Nuclear cystography has been utilized to reduce the radiation exposure for children during follow-up of VUR. Nuclear cystography, while being more sensitive, does not permit specific grading of VUR and does not reveal anatomical defects such as ureterocele, diverticulum, or the presence of posterior urethral valves. However, it is useful to determine if VUR has resolved after surgical correction.

Technetium 99 m Dimercaptosuccinic Acid Renal Scan (DMSA renal scan)

DMSA is currently the gold standard for diagnosing acute pyelonephritis and renal scarring (see ▶ Chap. 6, “Imaging Kidneys and the Urinary Tract”). Single-photon emission computed tomography (SPECT) DMSA scintigraphy is superior to planar imaging for the detection of renal cortical damage [128, 129]. DMSA scintigraphy is more sensitive than ultrasound, intravenous urography, or CT scan in diagnosing renal scarring [122, 123, 130]. The sensitivity of DMSA scintigraphy in experimentally induced acute pyelonephritis in a pig model was reported to be 92%, when compared to histological findings [131]. The RIVUR study revealed a significant interobserver variability in the reporting of abnormal DMSA renal scans compared with the previously published studies [132], which should be kept in mind when making clinical decisions.

While DMSA scans correlate with acute pyelonephritis, they are not recommended in the standard management of acute pyelonephritis. Cortical defects identified on a DMSA scan performed during or shortly after APN may be due to preexisting lesions (either acquired RN or congenital RN) or to the acute inflammatory reaction associated with acute pyelonephritis. A delay in performing the DMSA scan to at least 4–6 months post-UTI allows the acute inflammatory reaction to subside, at which point any persistent cortical defects are assumed to represent permanent renal scarring [133].

Jodal et al. have suggested the use of the DMSA renal scan as an initial study in the evaluation after diagnosis UTI in children [134]. This “top down” approach is an alternative to a VCUG after UTI, also called the “bottom up” approach. However, the DMSA scan results in more radiation exposure than the VCUG or the nuclear cystogram. Due to a shortage of Technetium 99^{m} dimercaptosuccinic acid, many centers are using 99^{m}Tc-glucoheptonate as the tracer and this is less sensitive for the detection of renal scars.

Magnetic Resonance Imaging (MRI)

MRI allows discrimination between areas of swelling from scarring, both of which look similar on DMSA scintigraphy (see ▶ Chap. 6, “Imaging Kidneys and the Urinary Tract”). MRI also allows the diagnosis of other coexisting conditions such as nephrolithiasis, which is not diagnosed by DMSA scintigraphy. However, routine use of MRI is less practical because of limited availability, the need for prolonged sedation, and the high cost.

Biomarkers of Renal Injury

Severe VUR may interfere with tubular function including urine concentrating abilities. Urine levels of N-acetylglucosaminidase (NAG) and urine osmolality have been examined to determine if they predict severe VUR. NAG is an enzyme produced by lysosomes within proximal tubular cells. Garcia-Nieto et al. assessed one hundred and sixty-three children and found that urine osmolality was significantly decreased in children with severe VUR even without evidence of renal scars when compared to children without VUR and children with low grade VUR. They also found that urine NAG was significantly increased in those children with severe VUR (higher than grade 3) when compared to urine NAG levels in children with mild VUR and those without VUR [135]. Procalcitonin levels in the blood have also been used to predict VUR. Procalcitonin is the prohormone of calcitonin and is an early and sensitive marker of bacterial infection. In infants less than 2 years of age who presented with a febrile UTI, an elevated procalcitonin level $\geq$1.0 ng/mL predicted both acute pyelonephritis and VUR identified by voiding cystourethrogram [136].

Numerous noninvasive biomarkers have been investigated for their usefulness in early diagnosis and monitoring of the progression of renal scarring. These include proteinuria, renal tubular enzymes such as NAG [137], renal tubular brush-border antigens (BBA) [138], epidermal growth factor [139], cytokines such as interleukin (IL)-8 [140] and IL-6, soluble tumor necrosis factor (TNF) receptor-1 [141], endothelin-1 [142], and prostaglandin E2 [143]. Low molecular weight proteins, such as beta 2-microglobulin (B2M), retinol-binding protein (RBP), alfa 1-microglobin (A1M), and lysozyme, pass freely through the glomerular basement membrane and are almost completely reabsorbed by the proximal renal tubule [144]. Their urinary excretion increases in proximal tubular damage, including that due to reflux nephropathy [69, 145]. An increased serum level of beta 2 microglobulin has also been associated with reflux nephropathy [146].

Proteinuria is a well-known predictor of renal disease progression due to RN [147]. Proteinuria is severe when associated with FSGS. A significant correlation exists between albuminuria and GFR [69]. In contrast to overt proteinuria, persistent excretion of small amounts of albumin, also called microalbuminuria, is helpful in diagnosing glomerular damage at a very early stage, as demonstrated in patients with diabetes mellitus [148]. In patients with RN, microalbuminuria has been reported around the same time as the appearance of abnormal levels of low molecular weight proteins in the urine [69, 145], and its excretion increases with increasing severity of VUR and renal scarring [149]. In a study of children with bilateral VUR with renal scarring and normal creatinine clearance, microalbuminuria was detected in 53.5% of the cases [66]. The diagnosis and monitoring of microalbuminuria offers the potential therapeutic possibility of using an angiotensin converting enzyme inhibitor (ACEI) and/or an angiotensin receptor blocker (ARB) to slow the progression of renal damage [150, 151].

Medical Management of VUR

For many decades, the management of VUR has been driven by the belief that VUR predisposes to recurrent UTI and renal parenchymal damage. Various treatment strategies have been used with the ultimate objective of preventing renal injury. The two main treatment modalities that have been practiced are long-term antimicrobial prophylaxis and surgical correction. The third option that has come up recently is surveillance with no routine prophylaxis or surgical intervention.

Antimicrobial Prophylaxis

The effectiveness of antimicrobial prophylaxis in the prevention of UTI recurrence has been studied extensively (see ▶ Chap. 55, “Urinary Tract Infections in Children”). The RIVUR trial revealed that trimethoprim-sulfamethoxazole (TMP/SMZ) prophylaxis reduced the risk of UTI recurrence by 50%. Similar results were reported by another placebo-controlled-double-blind (PRIVENT study) trial [152]. Combined results from the RIVUR and Careful Urinary Tract Infection Evaluation (CUTIE) studies revealed that toilet-trained children with VUR and BBD exhibit the greatest benefit from antimicrobial prophylaxis [153]. However, many other randomized studies have shown either no [154–156, 157, 158] or a female-restricted beneficial effect with prophylaxis [159]. Systematic reviews and meta-analyses have also reported mixed results, some concluding that prophylaxis is effective [160, 161] while others reporting that prophylaxis offers none or very little advantage for the prevention of UTI recurrence

[162, 163]. These variations in results have been attributed to significant differences in study designs, including patient inclusion and exclusion criteria [164]. No study has demonstrated any beneficial effect of antimicrobial prophylaxis for the prevention of renal scarring. However, none of these studies were powered to evaluate renal scarring as the primary outcome.

The antimicrobial agents most appropriate for prophylaxis include trimethoprim-sulfamethoxazole, trimethoprim alone, nitrofurantoin, or cephalexin (see ▶ Chap. 55, "Urinary Tract Infections in Children"). In view of increasing resistance of *E. Coli*, ampicillin or amoxicillin are less effective as prophylactic agents [165–167] and are not generally prescribed beyond the first 2 months of life. During the first 2 months of life, it is advisable to avoid TMP-SMZ because it may worsen hyperbilirubinemia and promote neurotoxicity. In toilet trained children, antibiotic prophylactic medication is generally administered at bed time, though this recommendation is not evidence-based. The prophylactic dose of antimicrobials is one-fourth to one-half of the therapeutic dose for acute infection. The dosage for commonly used antimicrobials for UTI prophylaxis is shown in Table 3.

Antibiotic resistance is a major risk of long-term prophylaxis and hence the latter should be used selectively. In cases with febrile UTI and VUR, the American Urological Association (AUA) recommends continuous antibiotic prophylaxis in children under 1 year of age and a selective approach in older children based on patient age, severity of VUR, recurrence of UTI, presence of BBD, and renal cortical anomalies [168]. The European Association of Urology/European Society of Pediatric Urology, the Swedish and the Italian Society of Pediatric Nephrology also recommend a more selective approach based on a combination of patient age, severity of VUR, and the presence of renal scarring [169–171]. Other factors that should be considered before initiating long-term antimicrobial prophylaxis include status of toilet training, risk of antibiotic resistance, anticipated compliance with daily medication administration, parental choice, and the medication expense. In all recommendations, younger age is a particular consideration for prophylaxis because infants may present with nonspecific clinical signs with a UTI. In addition, infants have risks for other potential complications including difficulty in getting urine specimens, a higher likelihood of hospitalization for IV antibiotic administration and hydration, an increased risk of septicemia, and greater family disruption and parental anxiety. In some cases, "preemptive" antimicrobial prophylaxis may be necessary to lower the risk of first UTI such as in those with high-grade VUR diagnosed during workup for antenatal hydronephrosis.

Table 3 Antimicrobial agents for urinary tract infection prophylaxis [188]

Antimicrobial agent	Dose
Trimethoprim (TMP)–sulfamethoxazole	2 mg TMP/kg/day
Nitrofurantoin	1–2 mg/kg/dose
Cephalexin	10 mg/kg/dose
Amoxicillin	10 mg/kg/dose

Secondary analysis of the RIVUR study revealed that patients who took TMP/SMZ $<70\%$ of the time were 2.5 times more likely to have a recurrent UTI (95%CI 1.1–5.6) and were at highest risk for renal scarring ($OR = 24.2$, 95%CI 3.0–197) as compared to more adherent patients [172]. The RIVUR study also revealed that long-term prophylaxis with TMP/SMZ was not associated with an increased risk of skin and soft tissue infections, pharyngitis or sino-pulmonary infections [173], or excessive weight gain [174]. Another study reported no abnormalities in blood chemistries, renal function, or complete blood count results in children receiving long-term TMP/SMZ prophylaxis for a duration of 2 years [175]. Regarding the cost-effectiveness of antibiotic prophylaxis, one study revealed that prophylaxis was associated with marginally higher costs as compared to placebo [176], while another study reported that prophylaxis was more cost effective than observation in children with high grade (grade IV), but not low grade VUR. These findings should be considered in light of the fact that the large majority of children with VUR do not have high-grade disease [177].

The duration of prophylaxis depends on multiple factors. Treatment duration may range from a few days until a VCUG can be obtained in those with a recently diagnosed UTI to a few years for children with known VUR and UTI risks. Other factors that impact the duration include the age of the patient, the severity of the VUR, the frequency of UTI, BBD, and the degree of renal scarring, if present.

Bladder Bowel Dysfunction

The appropriate management of BBD is necessary to prevent recurrence of UTI, to promote resolution of VUR and to prevent further renal injury. Treatment of constipation by dietary measures, behavioral therapy, and laxatives helps to reduce UTI recurrence and to treat enuresis (see ▶ Chap. 59, "Enuresis") and uninhibited bladder contractions. Timed, frequent, and complete voiding every 2–3 h is a crucial part of management. Pelvic floor exercises, biofeedback, and/or anticholinergic medication or alpha-blockers may be required to improve bladder function. Anticholinergic medications may however worsen constipation. Conservative medical treatment coupled with computer game-assisted pelvic floor muscle retraining can decrease the incidence of breakthrough UTIs and facilitate VUR resolution in children with voiding dysfunction and VUR. Continuous intermittent catheterization may be necessary in extreme cases, as seen in Hinman's syndrome where there is functional bladder outlet obstruction from a nonneurogenic neurogenic bladder (see ▶ Chap. 58, "Bladder Disorders").

Hypertension and/or Proteinuria

Appropriate management of hypertension and/or proteinuria is important to slow down the progression of renal disease. While many antihypertensive drugs exist, angiotensin converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) are preferred due to their renoprotective effect. Studies have revealed that in addition to lowering blood pressure, ACEIs reduce proteinuria due to reflux nephropathy. In a study by Lama et al [150], 15.5% of children with VUR and reflux nephropathy had microalbuminuria. Treatment with an ACEI (captopril) for 2 years was associated with decreased microalbuminuria. Of note, GFR, serum creatinine, and BP remained stable. Notably, it is not known whether this antiproteinuric effect slows down the progressive loss of renal function. In patients with a unilateral poorly functioning scarred kidney, surgical removal of the affected kidney may normalize blood pressure.

Surgical Management of VUR

Surgical correction of VUR is usually reserved for patients with high-grade VUR, recurrent UTI despite antibiotic prophylaxis, noncompliance or intolerance to prophylactic antibiotics, and/or the worsening of renal scars [178]. Open ureteral reimplantation remains the mainstay of surgical correction for VUR, although endoscopic treatment is also widely used, particularly for low grade VUR. Laparoscopic reimplantation techniques (with or without robotic assistance) have increased in recent years but utilization remains limited to specific centers. Endoscopic treatment involves subureteral or intraureteral injection of a bulking agent (most commonly dextranomer/hyaluronic acid (Deflux®). The decision for timing and type of intervention is based on the age of the patient, the status of the kidneys, the grade of VUR, and parental/patient wishes.

Endoscopic Management

Endoscopic treatment is performed under anesthesia in an outpatient setting and involves the injection of a bulking agent in a subtrigonal location at the ureterovesical junction in the bladder (STING procedure). With the use of cystoscopy, the ureteral orifice is identified and an endoscopic needle is utilized to inject material under the ureteral orifice in the submucosal space.

Currently, endoscopic treatment of reflux with dextranomer hyaluronidase (Deflux®) is used by many as the first-line surgical treatment in children with low grades of VUR. Meta- analysis of all of the endoscopic modalities used for management of VUR indicates that rates of cure per ureter for grades I and II were 78.5%, grade III were 72%, grade IV were 63%, and grade V were 51%. Success rates of 68% were noted for those patients failing first injection and 34% for those failing a second injection. Reduced success rates were noted for those patients with duplicated systems and those with neurogenic bladders [179]. The success rates and the minimally invasive nature of endoscopic procedures have led to a substantial increase in the number of procedures being performed.

A systematic review revealed a success rate of 77% at 3 months after a single injection. Efficacy ranged from 81% for grade I VUR to 62% for grade V VUR [180]. Another study with 5 years of follow-up reported success rates of 68% [181]. Fortunately, no one has reported particle migration with the use of dextranomer/hyaluronic acid copolymer beads and the procedure is being used in complex urological conditions including ureteroceles, duplicated systems, and even failed open reimplants.

Rarely, postoperative ureteral obstruction has been noted in patients following injection therapy. A higher incidence of this complication has been noted in children with voiding dysfunction and a neurogenic bladder. Calcification of the implant of dextranomer/hyaluronic acid can resemble a distal ureteral calculus. Some patients have been misdiagnosed as having a possible obstructive distal ureteral stone when in fact they had an asymptomatic remnant of the bead that caused this appearance on ultrasound.

Laparoscopic Ureteral Reimplantation

Laparoscopic ureteral reimplantation has the benefit of providing results that are comparable to open surgery, with the promise of a minimally invasive approach. The procedure is typically performed using a transperitoneal approach and reimplantation is performed using an extravesical technique. Laparoscopic reimplantation has been associated with long operative times and high cost when disposable equipment is utilized. Additionally, appropriate patient selection is critical for surgical success using laparoscopy. Ureteral injury has been noted with this procedure and is related to the steep learning curve associated with complex laparoscopy. Additional complications that have been noted include ureteral leak in 12.5% and ureteral stricture in 6.3% [182]. Overall success with correction of VUR is similar to the open procedures [182]. This procedure has gained wider acceptance with the use of operative robots that permit greater ease of intracorporeal suturing.

Open Surgical Techniques

The gold standard for the surgical management of VUR remains the open approach. The most commonly performed open technique is the Cohen cross-trigonal reimplantation. This procedure has wide applicability and can be performed even in the presence of duplicated systems and other intravesical abnormalities. The success rate of this procedure for the correction of reflux is 98% [183]. Indeed, many clinicians have stopped performing follow-up VCUG studies to confirm the resolution of reflux after this procedure. Improvements in surgical anesthesia and postoperative pain management have allowed children to be managed with a 24- to 48-h hospitalization. Some children have even been managed as outpatients.

If residual VUR is noted following surgical reimplantation, continued monitoring of the patient and repeat VCUG testing 3–4 months later may show further resolution. Most children that have residual VUR following open surgery have a lower grade of VUR than their original grade. Contralateral VUR is noted in 19% of patients that have had unilateral ureteral reimplantation [184]. The majority resolve over the ensuing 2 years and are felt to be secondary to trigonal changes following ureteral reimplantation. Ureteral stricture has also been reported and is secondary to ischemia of the distal ureter. Endoscopic access to the ureter following ureteral reimplantation can be difficult and therefore later management of stones may be difficult.

Published Guidelines on UTI/VUR

Many guidelines have been published on the management of UTI and/or VUR. These include the Swedish [171], National Institute for Health and Clinical Excellence (NICE), United Kingdom

[185], American Academy of Pediatrics (AAP) [186], the recently updated American Association of Urology (AUA) [168], and the Italian Society of Pediatric Nephrology (ISPN) [187] guidelines (see ▶ Chap. 55, "Urinary Tract Infections in Children"). None of the recommendations advise a routine VCUG after the first febrile UTI. NICE and the AAP do not recommend routine antimicrobial prophylaxis, whereas the Swedish, ISPN, and the AUA recommend a selective use of antibiotic prophylaxis. None of these recommendations support the use of the DMSA renal scan in the acute management of febrile UTI.

Conclusion

The emerging knowledge about VUR has generated a lot of interest among clinicians as well as researchers. Clinical and possibly genetic differences have been noted between VUR diagnosed during sibling screening, during follow-up for antenatal hydronephrosis or after UTI. Additionally, the role played by bladder and bowel dysfunction and/or chronic constipation in the resolution of VUR and the frequency of UTI have not been completely elucidated. Prevention of renal scars in children with VUR and the preservation of renal function in those with renal scars remains the most important objective. The current management of VUR focuses on the prevention of UTI, with curative surgery limited primarily to children that fail conservative measures. Antimicrobial prophylaxis reduces the recurrence of UTI but its role in the prevention of renal scarring remains to be determined. A better understanding of gene mutations that lead to VUR and recurrent UTI and/or renal scarring is likely to help resolve some of the challenges faced in the management of VUR and prevent renal injury.

Cross-References

- ▶ Bladder Disorders
- ▶ Congenital Anomalies of the Kidneys
- ▶ Imaging Kidneys and the Urinary Tract
- ▶ Pediatric Obstructive Uropathy
- ▶ Pre-natal Development of the Kidneys and Urinary Tract
- ▶ Urinary Tract Infections in Children

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Pediatric Obstructive Uropathy

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Abstract

Congenital obstructive uropathy, a major cause of chronic kidney disease, is defined as kidney, ureter, and/or bladder injury associated with obstruction of the urinary tract. Congenital obstructive uropathy arises from malformation of the renal collecting system, constituted by the calices, pelvis, and ureter, the bladder and urethra, and the connections between the ureter and pelvis and the ureter and bladder. Congenital obstructive uropathy is caused by a polymorphic group of unilateral or bilateral upper and lower urinary tract malformations. Presenting most commonly as antenatal hydronephrosis, these disorders range in severity from self-resolving pelviectasis and/or hydroureter to a fixed physical obstruction of the ureteropelvic junction, ureterovesical junction, or urethra causing kidney injury. In this chapter, the clinical assessment of urinary tract obstruction and the pathobiology of obstructive uropathy-associated kidney injury are described from observations in animal models and descriptive analyses of human kidney tissue. Genes and genetic pathways that control the embryonic development of the ureter and its connections with the bladder are highlighted as well genetic mutations associated with malformation of the ureter, ureteropelvic junction, ureterovesical junction, and urethra. A clinical classification of antenatal urinary tract dilatation is presented; for each specific disorder, genetic etiologies, diagnosis, use of static and dynamic imaging modalities, natural history, and treatment approaches are described.

Keywords

Obstructive uropathy · Ureteropelvic junction obstruction (UPJO) · Ureterovesical junction obstruction (UVJO) · Posterior urethral valves (PUV)

Synonyms

Obstructive uropathy

Introduction

Congenital obstructive uropathy is a heterogeneous group of pathologies with a varying natural history. Obstruction of the urinary tract can be at the level of the ureteropelvic junction, the ureterovesical junction, the bladder outlet, or urethra. It can be either unilateral or bilateral and it may involve some or all of the urinary tract. Congenital obstructive uropathy represents a major cause of renal failure in infants and children. Together with renal hypoplasia and dysplasia, it accounts for almost half of all cases of chronic kidney diseases in children.

Epidemiology

Urinary tract abnormalities identified by ultrasound are one of the most common anomalies with a rate of 1:250 to 1:1000 pregnancies [1]. Congenital obstructive uropathies account for most of these cases: approximately 1:2000 pregnancies. The mean prevalence of all types of renal malformation is 1.6 per 1000 births. The most frequent diagnosis is hydronephrosis with more than 80% being detected prenatally [2, 3]. Unilateral hydronephrosis is more common than bilateral, and most cases are mild and will resolve spontaneously. The differential diagnosis of hydronephrosis includes ureteropelvic junction obstruction, vesicoureteral reflux, ureterovesical junction obstruction, posterior urethral valves, prune belly syndrome, urethral atresia, and duplicated collecting system with ureterocele. Ureteropelvic junction obstruction is the most frequent cause of hydronephrosis (reported incidences of 39–64%), followed by vesicoureteral reflux (33%) and ureterovesical junction obstruction (9–14%). The incidence of lower urinary tract obstruction is 2.2 per 10,000 newborns with posterior urethral valves being the most common malformation at 64% (1.4:10,000 births), followed by urethral atresia at 39% (0.7:10,000 births) and Prune belly syndrome (Eagle-Barratt syndrome) (4%) [4].

Definition of Obstruction

Whitaker et al. described urinary tract obstruction as any condition “that impairs urinary drainage from the pelvicalyceal system and leads to increased pressure and reduced urine flow rate” [5]. This was the first approach to define obstructive uropathy from a functional perspective. Koff et al. added potentially endangered renal function to impaired urinary drainage in the definition of obstruction, resulting in “any restriction to urine flow that will cause progressive renal deterioration” [6]. Peters et al. created the most recent and generally accepted definition describing obstruction as “any condition that, left untreated, endangers renal function and growth potential” [7]. Clinically, obstruction is usually presumed when urinary tract dilatation (i.e., hydronephrosis) is detected by ultrasound. But not every patient with hydronephrosis has an obstruction (or significant obstruction) as most cases of antenatal hydronephrosis will improve without surgical intervention or renal damage. It is important to state that hydronephrosis *per se* does not correlate with the affected kidney’s function. Hydronephrosis is problematic when it endangers renal function. At the time of diagnosis, some patients will have irreversible renal damage, whereas others will have progressive renal injury if left untreated. It is still a challenge for physicians to identify and categorize this heterogeneous group of obstructive pathologies.

Pathophysiology

The urinary tract consists of conduits and reservoirs transporting the urine produced by the kidneys. To better understand the pathophysiology of obstructive uropathy, the properties of this system have to be considered, including structural compliance, flow impairment, renal function, and urine production. It is important to understand that the onset and the duration of obstruction correlate with abnormal kidney development and loss of kidney function. In fetal development, urinary tract obstruction impairs renal growth and development. It is therefore fundamentally distinct from obstruction acquired later in life. The development of congenital obstructive uropathy is regulated by a complex interplay of genetic and nongenetic factors, only some of which have been identified [8–12].

Animal Models

Congenital obstructive uropathy initiates a complex sequence of events resulting in impaired renal function. Its pathophysiology has been studied intensively, leading to the development of multiple animal models, including complete and partial ureteral obstruction, unilateral and bilateral obstruction, and acute and chronic obstruction [8, 9]. Experimental obstructive uropathy is usually created by unilateral ligation of the ureter. Advantages of this surgical model include experimental manipulation of the severity, duration and timing of obstruction, as well as the study of recovery following release. The unilateral ligation of the ureter induces progressive tubular cell injury in the kidney, including apoptosis, necrosis, and necroptosis [13], reduced proliferation, loss of differentiation, and tubular atrophy. Ureteral obstruction also leads to interstitial inflammation, interstitial fibrosis, and extracellular matrix deposition. Experimental obstructive uropathy induces glomerular and vascular hemodynamic changes that are different in neonatal mice and rats when compared to adult rodents in which nephrogenesis is complete.

Tubular Injury After Ureteral Obstruction

Early changes observed after ureteral obstruction consist of tubular dilatation, which is mostly pronounced in the collecting ducts. The mechanical stretch of tubular cells induces progressive tubular apoptosis accounting for significant loss of renal tissue. A variety of molecules mediate these tubular changes, including those related to apoptosis and cell cycle regulation (caspases, intrinsic and extrinsic death pathway molecules, reactive oxygen species, catalase, and inhibitors of cyclin-dependent kinases p27 and p21) [9]. Upon injury, some tubular segments lose epithelial markers (E-cadherin) and gain de novo-expression of mesenchymal cell markers (α-smooth muscle actin, vimentin), suggesting transformation/dedifferentiation of tubular epithelial cells into mesenchymal cells or epithelial-mesenchymal transformation [14, 15]. Ureteral obstruction also leads to glomerulotubular disconnection, resulting in atubular glomeruli and aglomerular nephron segments [16]. The functional tubular changes observed after ureteral obstruction include impaired reabsorption of solutes and water, and reduced capacity to concentrate and acidify the urine [17].

Interstitial Inflammation After Ureteral Obstruction

Unilateral ureteral obstruction induces recruitment and infiltration of inflammatory cells into the kidney. This inflammatory infiltrate consists predominantly of macrophages and activated T-lymphocytes, which together with injured tubular cells release chemokines, proinflammatory and profibrotic cytokines, and reactive oxygen species. Neutrophils are rarely found in obstructed kidneys. In addition to producing cytokines, infiltrating leukocytes also release chemokines (e.g., CCL2) and express chemokine receptors (e.g., CCR1, CCR5) as well as other receptors (Toll like receptor-2), activating both paracrine and autocrine loops for macrophage and T-cell recruitment. Multiple chemokines, chemoattractants, and adhesion molecules regulate the interstitial inflammation (CCL2, osteopontin, IL-1, VCAM-1, ICAM-1, angiotensin II, selectins, β2-integrins, and receptor for advanced glycation end products – RAGE) [18]. Different macrophage subpopulations, some harmful and others protective, have been found in the kidney after unilateral ureteral ligation and may be important targets for future therapies [19].

Interstitial Fibrosis After Ureteral Obstruction

Experimental ureteral obstruction eventually leads to interstitial fibrosis, characterized by the activation of interstitial fibroblasts, the production and deposition of extracellular matrix and the loss of peritubular capillaries in the obstructed kidney. Increased fibroblast density in the kidney was traditionally explained by the local proliferation of resident fibroblasts. Recently, it has been shown that this mitogenic process accounts for only about half of interstitial fibroblasts. Other mechanisms such as recruitment of bone marrow-derived fibrocytes from the circulation and epithelial to mesenchymal transformation of tubular cells may contribute to the increased number of interstitial renal fibroblasts [14, 15, 20]. Pericytes and endothelial cells have been identified as additional sources of interstitial fibroblasts [21]. Various molecules drive these interstitial changes like the regulators of epithelial to mesenchymal transformation (hepatocyte growth factor, bone morphogenic protein 7, TGF-β1, nestin, and HIF1-α) [22], hypoxic injury responses, cytokines, and growth factors (TGF-β1, EGF, VEGF, IGF-1, PDGF-C, connective tissue growth factor and TNF-α) [9]. These changes collectively induce progressive scarring, and the loss of renal tissue and kidney function. Extracellular matrix proteins contributing to fibrosis in the obstructed kidney include collagen types I, II, III, IV, V, VII, and XV; fibronectin, laminin, and heparin sulfate proteoglycans.

Glomerular, Vascular and Hemodynamic Changes After Ureteral Obstruction

In adult animals with ureteral obstruction, glomerular and vascular changes are observed in advanced stages. Nonspecific glomerular changes including capillary wall thickening or collapse, global sclerosis, and vascular intimal thickening reflect chronic ischemia of the obstructed kidney. In contrast to the adults with obstruction, glomerular changes are much more pronounced and occur earlier in neonatal animals [23]. Renal blood flow, glomerular filtration rate, and tubular function change immediately after ureteral obstruction. After a brief period of hyperemia and transient increase in renal blood flow, a progressive and permanent vasoconstriction leads to a decline of renal perfusion and glomerular filtration rate. Vasoconstrictive agents like angiotensin II, thromboxane A2, and prostacyclins in combination with a reduced production of vasodilating substances including nitric oxide, prostaglandins, and platelet-activating factor regulate these functional changes [9].

Renal Injury in Neonatal Ureteral Obstruction

Most preclinical studies to investigate the pathophysiology of ureteral obstruction have been performed in adult animals. Few studies have been conducted in fetal sheep and monkeys or in neonatal rodents (mice and rats). In humans, nephrogenesis is complete at 34–36 weeks of gestation [24] and thereafter only functional maturation occurs. In contrast, in rats and mice only 10% of mature glomeruli are generated at birth, with the remaining nephrons formed postnatally. Based on the duration of nephrogenesis, the neonatal rat or mouse parallels the mid-trimester human fetus [9]. This is the time when renal obstruction usually starts in the human system. Thus, ureteral obstruction experimentally conducted in the newborn mouse offers a suitable animal model to investigate obstructive uropathy.

Moreover, pioneering work by Chevalier and his coworkers established a model of neonatal obstructive nephropathy in newborn mice and rats at the second day of life. Their technique of unilateral ureteral obstruction allows for the study of urinary blockage during kidney development [25]. Complete unilateral ureteral obstruction in neonatal rodents results in arrested renal development, renal dysplasia, fewer glomeruli, and reduced glomerular surface area; it impairs growth and maturation, and ultimately reduces the number of nephrons [23]. The severity of renal injury depends on the degree and the duration of obstruction. Neonatal ureteral obstruction for four days (and release thereafter) reduces renal growth by 60% and the number of glomeruli by 50% [26]. Neonatal ureteral ligation leads to hemodynamic changes, tubular atrophy, interstitial fibrosis, and abnormal development of the renal vasculature. Similar histologic lesions were found in kidney biopsies of children obtained during pyeloplasty [27]. Some of the underlying pathways driving interstitial inflammation, tubular apoptosis, and interstitial fibrosis in neonatal ureteral obstruction have been identified (Table 1, modified from [28]).

For lower urinary tract obstruction, the fetal sheep has been the most used model, with the majority of work done by Harrison and colleagues. Using this model, the effect of ureteric and urethral

Table 1 Pathways for obstructive renal injury

Apoptosis	Inflammation	Fibrosis
Hypoxia, Ischemia	Angiotensin II	TGF-β
Reactive oxygen species	TGF-β	Angiotensin II
Caspases	Chemokines (CCL2, CCL5)	EMT / EndMT
Cellular stretch	Adhesion molecules (Selectins, β2-Integrins), RAGE	Activated Myofibroblasts
EGF	Macrophage infiltration	ECM, α-SMA
TNF-α	NF-κB	SMAD2, SMAD3

Legend: Pathogenesis of obstructive uropathies. There are three major pathways for obstructive renal injury: apoptosis (and other forms of cell death), interstitial inflammation, and eventual interstitial fibrosis. Epidermal growth factor (EGF), Tumor necrosis factor-α (TNF-α), Transforming growth factor-β (TGF-β), Receptor for advanced glycation end products (RAGE), Nuclear factor-κB, (NF-κB), Epithelial-mesenchymal transition (EMT), endothelial-mesenchymal transition (EndMT), Extracellular matrix (ECM), and α-smooth muscle actin (α-SMA). Table modified from [28] obstruction, both partial and complete, and its effects on pulmonary and renal development has been identified [29]. Complete urethral obstruction produced severe hydronephrosis, megaureter, megacystis, and pulmonary hypoplasia, but not cystic and/or dysplastic changes in the kidney. In contrast, complete unilateral ureteral obstruction in the fetal lamb induced dysplastic kidneys. These histologic lesions were similar to those seen in the human fetus [30]. In addition, in-utero decompression either prevented or greatly improved renal dysplasia [31]. Other animal models have reproduced the finding of dysplastic kidneys with ureteral obstruction [31].

Unfortunately, interstitial fibrosis and irreversible renal damage, which are present in patients with poor kidney function, rarely improve after surgical correction. Therefore, the focus of most studies has more recently shifted to the identification of novel biomarkers to characterize and identify obstructive uropathies before permanent damage has occurred [32].

Genetics

Experimental studies to identify genetic defects in patients with obstructive uropathy have provided an enormous wealth of data. The mouse has been instrumental in further characterizing putative genes involved in obstructive uropathy. The strong similarities between the human and the mouse genome together with genetic engineering technologies provided excellent research tools to study the effects of human mutations *in vivo* [32, 33]. Although only a few causative genes associated with obstructive uropathy in humans have been discovered so far, basic research in this area gave important insights on early urinary tract development (Table 2). Most cases of kidney and urinary tract anomalies are sporadic and restricted to the urinary tract [24]. The majority of sporadic cases cannot yet be explained by monogenic causes. Only some subsets of "congenital anomalies of the kidney and urinary tract" (CAKUT) are part of a syndrome or are associated with a positive family history [1, 24]. To date, 40 genes have been identified as monogenic causes of isolated CAKUT and approximately 150 genes have been identified for syndromic CAKUT [34–36].

Genes and Signaling Pathways in Human and Mice

Many genes and signaling pathways have been identified to play important roles in early ureteric budding and ureter development [37]. These include the renin-angiotensin system (e.g., *Agt, Ren, Agtr1, Agtr2*), the receptor tyrosine kinase (RTK) signaling pathway (e.g., *Gdnf, Ret, Fgfr2*), the Wnt signaling pathway (e.g., *Ctnnb1, Wnt7b, Wnt9b, Fzd1*), the Hedgehog signaling pathway (e.g., *Shh, Gli3, Smo, Tshz3*), the TGF-β signaling pathway (e.g., *Bmp4, Smad4*), and the retinoic acid-mediated nuclear receptor signaling pathway (e.g., *Rara, Rarb*). Urinary tract diseases can modify the expression and activity of these genes and pathways [38].

Genetic studies in both human and mice have identified many genes associated with hydronephrosis due to urinary tract abnormalities [36, 39] (also see ▶ Chap. 1, "Pre-natal Development of the Kidneys and Urinary Tract"). Antenatal hydronephrosis can originate from kidney defects (e.g., mutations in *AQP2* that encodes the aquaporin 2 water channel in the collecting tubule) [40], or from defects in the ureter such as vesicoureteral reflux or from urinary tract obstruction [41, 42]. Mutations in many genes controlling the developmental process of the ureter have been shown to cause hydronephrosis in mice [39]. In addition, mutations in several murine genes have been shown to cause ureteropelvic junction obstruction and ureter-bladder connections defects [43–45]. Loss of both retinoic acid receptor alpha (*Rara*) and beta (*Rarb*) in mice lead to megaureter and hydronephrosis due to abnormal apoptosis mediated by vitamin A signaling at the ureter-bladder insertion site [43]. Deletions of uroplakins (*Upk3a* and *Upk2*) in the urothelium in mice cause loss of the superficial cell layer, overgrowth of the urothelium, urothelial leakage, and vesicoureteral reflux [46]. Stromal cells are also important for ureteral development and function [47]. Loss of Disc-large homolog 1 (*Dlg1*) causes absence of the stromal cell layer in the ureter, which leads to abnormal ureteral smooth muscle cell orientation, impaired ureteral peristalsis, and severe antenatal hydronephrosis [47]. *Dlg1* is the only gene identified so far that is required for ureteral stromal cell formation. Many genes associated with hydronephrosis in mice are genes controlling ureteral typical smooth muscle cell development [48]. These include genes in the sonic hedgehog pathway (e.g., *Shh, Gli3, Smo, Tshz3*) [49, 50], the TGF-β pathway (e.g., *Bmp4, Smad4*) [51, 52], and the Wnt pathway (e.g., *Ctnnb1*) [53]. In mice, loss of the T-box transcription factor *Tbx18* induces failure of the ureteral mesenchymal cells to differentiate into ureteral smooth muscle cells as well as an abnormal differentiation of the urothelium, leading to ureterovesical junction obstruction, short hydroureter, and antenatal hydronephrosis [54]. The abnormal smooth muscle phenotype in *Tbx18* mutant mice may be caused by the downregulation of the sonic hedgehog signaling (e.g., *Ptch1*) in the ureteral mesenchyme and lower *Bmp4* expression in the ureter [54]. These results underline the importance of typical smooth muscle cells in the ureter, which produce contractile forces to transport urine from the kidney to the bladder [55]. Different genes control the development of ureteral pacemaker cells and the peristalsis machinery [48, 50, 56, 57]. *Hcn3* (hyperpolarization-activated cation channel 3) plays a fundamental role coordinating proximal-to-distal ureter peristalsis [57]. Inactivation of *Smo* (Smoothened) and upregulation of the GLI3 repressor, two members of the sonic hedgehog signaling pathway, lead to abnormal ureteral peristalsis, nonobstructive hydronephrosis, and hydroureter in mice [50]. Although the urothelium and smooth muscle cells develop normally in these mutant mice, the number of ureteral pacemaker cells is significantly reduced. Mice with defective pacemaker cell differentiation show abnormal ureteral peristalsis and hydronephrosis [50, 58]. Consistent with these findings, mutations in *Gli3* gene have been identified in patients with the Pallister-Hall syndrome, which includes urinary tract phenotypes like hydronephrosis and hydroureter (Table 2) [59]. In the murine urinary tract, mesenchymal expression of one of the calcineurin subunit B isoform genes called *Cnb1* (also named *Ppp3r1*) is required for the development of the pyeloureteral peristalsis [56]. Tissue-specific knockout of *Cnb1* in mice causes an abnormal formation of the renal pelvis and ureter as well as defective peristaltic waves, which lead to progressive renal tract obstruction and hydronephrosis after birth [56].

The angiotensin type 1 receptor (*Agtr1*) plays an important role in ureteral peristalsis. *Agtr1* knockout mice have an abnormal renal pelvis and lack ureteral peristaltic waves, and subsequently develop a hydronephrosis phenotype [60]. However, no causative mutations in either *CNB1* or *AGTR1* have been reported in patients with urinary tract anomalies although mutations in *AGTR1* are associated with autosomal recessive renal tubular dysgenesis (RTD) [61]. Molecular mechanisms leading to hydronephrosis and hydroureter in the absence of

Table 2 Genes associated with hydronephrosis and/or hydroureter (no VUR) in human

Gene	Chr	Type of protein	Signaling	Human disease (OMIM)
AGTR2	Xq22-q23	G-protein coupled angiotensin II receptor	RAS	X-linked mental retardation-88 (OMIM 300852)
WFS1	4p16.1	Transmembrane protein		Wolfram syndrome (OMIM 222300): hydronephrosis, dilated ureters, distended bladder without VUR
FOXC1 FOXF1	6p25	Forkhead transcription factor	Foxc	Axenfeld-Rieger syndrome type 3 (OMIM 602482): UPJO
GLI3	7p13	Zinc finger	Hedgehog	Pallister-Hall syndrome (OMIM 146510)
RET	10q11.21	Receptor	RTK	Hydronephrosis, megaureters, renal dysplasia
STRA6	15q24.1	A receptor for retinol/retinol-binding protein complexes	Retinoic acid signaling	Hydronephrosis

Abbreviations: Chr: chromosomal location; Signaling: signaling pathway; RAS: Renin-angiotensin system. Table modified from [39] anatomic obstruction include T-box transcription factor 18 (Tbx18) or SIX-1-dependent delay in differentiation of ureteral smooth muscle cells [54, 62]. The Rearranged in Transfection (RET) tyrosine kinase signaling system is the major pathway guiding the normal development of the kidneys, ureters, peripheral, and enteric nervous system. In the kidneys, RET is activated by interaction with the ligand glial cell line-derived neurotrophic factor (GDNF) and its coreceptor GFRα1. This activated complex regulates a number of downstream signaling cascades that control proliferation, migration, and apoptosis. RET mutations are found in 5–30% of CAKUT patients [63], and a number of *Ret* mouse mutants show a spectrum of kidney and urinary tract defects reminiscent of CAKUT in humans [64]. Both, loss and overexpression of RET can cause urinary tract anomalies. Recently, it has been shown that in mice with a mutation in the RET-PLCγ docking site (Y1015), the common nephric ducts (CNDs) do not undergo timely degeneration and fail to separate from the Wolffian duct (WD), resulting in obstructive uropathy [65]. The ureter defect in RETY1015F mice is different from that in RET-null mice, as it manifests during the ureter maturation stage after the Wolffian duct has reached the cloaca. A phenotype similar to Y1015F ureter defects (ureterovesical junction obstruction) was shown in mice deficient for leukocyte antigen-related (LAR) family protein tyrosine phosphatase, receptor type S and F (PTPRS and PTPRF) [45]. These ureter-bladder connection defects cause urinary obstruction, hydroureter, and hydronephrosis in *Ptprs Ptprf* double deficient embryos [45]. Increased activation of RET-docking tyrosines in these mice implies an interaction between LAR and RET signaling pathways. Sprouty protein (SPRY1) inhibits RET tyrosine signaling by suppressing RAS-MAPK activity. Therefore, a *Spry1*-null mutation causes hyperactivation of the RAS-MAPK cascade. *Spry1*-null mice show multiplexed dysplastic kidneys with megaureters and an increased number of ureteric buds. Double knockout mice lacking RET-PI3K-MAPK signaling and *Spry1* show morphological correction of the renal anomalies in contrast to the individual null mice [66]. The mechanism for normalization of the renal defects was explained by an increase in RAS-MAPK signaling via a pathway other than RET. A possible candidate for this was FGFR signaling as in the absence of GDNF/RET signaling FGF7 can induce ureteric buds. Support for alternative tyrosine kinase activity came from compound homozygous mice lacking *Gdnf* or *Ret*, and *Spry1*. Deleting *Fgf10* from *Ret-Spry1* double nulls resulted in no ureteric buds, suggesting that FGF10 was the alternative RTK signal for ureteric bud induction [67].

Kidney and urinary tract development is sensitive to precise timing and to the level of MAPK activity. This activity can be altered by the dosage of the RET signaling complex, or through activation of key docking tyrosine of RET, or by interactions with negative regulators such as SPRY1 [64]. The GATA-RALDH2-RET molecular network plays a fundamental role in guiding the correct insertion of the nephric duct into the mouse bladder [44]. Absence of *Ret*, *Gata3*, or *Raldh2* causes similar defects of ureter insertion with urinary tract obstruction and hydronephrosis in mice [44].

Gene expression may also be regulated at the posttranscriptional level by noncoding microRNAs (miRNAs). A critical role for miRNAs in kidney development is evident from the observation that targeted genetic inactivation of Dicer, the miRNA-processing enzyme, in ureteric bud in mice results in hydronephrosis [68].

The broad phenotypic spectrum of obstructive uropathy and the variability in genotype–phenotype correlation indicate that its pathogenesis is a complex process depending on many factors. Derangements in cellular, molecular, and morphogenetic mechanisms that direct normal renal system development are important causes of congenital genitourinary disorders.

Genetics and the Renin-Angiotensin System

The renin-angiotensin system (RAS) plays a major role in controlling blood pressure, fluid, and electrolyte homeostasis. The developing mammalian kidney expresses all components of the RAS as early as the 5th week of gestation, when metanephric organogenesis is initiated [69]. Mutations in the genes encoding components of the RAS in mice cause diverse forms of congenital kidney and urinary tract malformations (CAKUT), which include hydronephrosis, hypoplastic medulla and papilla, thickening of renal arterial walls, duplex ureters, and vesicoureteral reflux [70]. The mechanistic basis of CAKUT due to RAS mutations involves hypoplastic ureteral smooth-muscle layer with impaired ureteral peristalsis [60]. Association of a single polymorphism in the human AT_2R gene (1332A>G transition) with CAKUT have been reported in several, but not all, studies. This alteration in the AT_2R is described in American and German Caucasian males with ureteropelvic junction obstruction, megaureter, and multicystic dysplastic kidneys [71]. The same polymorphism was detected in Korean children with unilateral multicystic dysplastic kidney, renal agenesis, and hydronephrosis [72], in Italian children with vesicoureteral reflux, hypoplastic kidneys, ureteropelvic junction obstruction, nonobstructive megaureter, and posterior urethral valves [73], and in Serbian children with ureteropelvic junction obstruction, vesicoureteral reflux, or megaureter [74]. In contrast, a large European study published by Cordell et al. found no association of primary vesicoureteral reflux with mutations in AT_2R [75]. This difference may be explained by limitations in study design, low penetrance of the mutation, or contribution of modifier genes. ACE polymorphism is also associated with kidney hypodysplasia caused by posterior urethral valves [76]. Collectively, these observations indicate that the pathogenesis of obstructive uropathy is a complex process that depends on the interplay of many factors. A concerted effort to provide new insights into pathologic processes is in progress within the Genitourinary Development Molecular Atlas Project (GUDMAP). Genetic counseling is recommended for all patients with familial cases of urinary tract malformations or newly diagnosed forms of CAKUT that suggest presence of genetic anomalies.

Classification

Table 3 shows the differential diagnosis of prenatally detected urinary tract dilatation. Idiopathic antenatal hydronephrosis (IAHN), defined as antenatal hydronephrosis not associated with other morphologic renal tract abnormalities, is the most common hydronephrosis phenotype detected by antenatal ultrasound [77]. Less common causes of hydronephrosis and, generally with greater pathologic significance, include ureteropelvic junction obstruction, ureterovesical junction obstruction, ectopic ureteral insertion with and without duplex system, posterior urethral valves, Eagle-Barratt syndrome, urethral atresia, and duplicated collecting system with and without ureterocele. Many of these prenatal dilatations will improve without surgical intervention or evidence of impaired kidney function. Nonobstructive causes of urinary tract dilation like vesicoureteral reflux must also be considered. The causes of obstruction can be classified on the anatomical level of obstruction (renal, ureter, bladder, or urethra) or on the underlying pathology (neoplastic, traumatic, inflammatory). Obstructive uropathies can be clinically classified as symptomatic or asymptomatic. Infants and children may present with febrile urinary tract infection, hematuria, a palpable flank mass, vomiting, or unexplained abdominal pain. However, with the use of antenatal ultrasound, symptomatic presentation has become less frequent. Occasionally, patients may show multiple abnormalities, for example, ureteropelvic junction obstruction and vesicoureteral reflux, which can be

Table 3 Causes of antenatally detected urinary tract dilatation

Causes of antenatally detected urinary tract dilatation
Idiopathic antenatal hydronephrosis
Ureteropelvic junction obstruction
Ureterovesical junction obstruction
Posterior and anterior urethral valves
Ectopic ureteral insertion w/o duplex system
Ureteral stricture
Retrocaval and retroiliac ureter
Ureterocele w/o duplex system
Vesicoureteral reflux
Prune belly syndrome
Extrinsic compression of the urinary tract
Neurogenic dysfunction, Megacystis microcolon intestinal hypoperistalsis syndrome
Complex anatomical abnormalities (cloaca, cloacal exstrophy)

difficult to discern. It is important to identify patients with involvement of both kidneys (bilateral ureteropelvic junction obstruction or ureterovesical junction obstruction), patients with a solitary kidney, or with poor function in the contralateral nonobstructed kidney. These children do not have the advantage of reserve functional kidney tissue and often benefit from surgical intervention. Poor excretion on nuclear scan could result from either poor kidney function or obstruction and as such can be difficult to identify those kidneys with obstruction. Under these circumstances, serial ultrasound examinations or magnetic resonance imaging (MRI) may be the best available indicator for surgical intervention.

Diagnosis

In the clinical setting, obstructive uropathy is assumed when dilatation, defined as an increased diameter of one or more components of the renal collecting system, is detected. Unfortunately, this dilatation does not necessarily correlate with the presence or degree of urinary flow impairment. Nonobstructive conditions can show similar findings (e.g., vesicoureteral reflux), and hydronephrosis *per se* does not correlate with kidney function. It is important to state that urinary tract dilatation also depends on the level of hydration. The fetal kidneys produce four to six times more urine before delivery than after; this increased volume may cause dilatation of the renal pelvis, at least transiently in early postnatal life [78]. All imaging techniques (ultrasound, nuclear scan, magnetic resonance urography) pre- and postnatally require sufficient hydration to depict and categorize urinary tract obstruction.

Prenatal Diagnosis

The majority of congenital abnormalities of the urinary tract are diagnosed during the second trimester ultrasound scan, which is usually performed at 18–20 weeks of gestation. However, with the increasing use of first trimester ultrasound screening, more severe renal abnormalities are being noted between 11 and 14 weeks [79]. The findings of prenatal ultrasound depend on the level and severity of the obstruction (Table 4). Unfortunately, no prenatal diagnostic test is available, which differentiates between obstructive and nonobstructive causes of hydronephrosis. Ultrasonography can detect increased echogenicity and cysts in the fetal kidney, which are suggestive of renal dysplasia; it can determine amniotic fluid volume, associated anomalies, and fetal sex. Predictors of outcome in lower urinary tract obstruction that can be identified by ultrasound include the gestational age at diagnosis, oligohydramnios, and kidney appearance. Detection of lower urinary tract obstruction (e.g., posterior urethral valves) before or at 24 weeks of gestation, oligohydramnios, and evidence of renal cortical cysts has been shown to predict poor outcome. Ultrasonography directs diagnostic procedures (amniocentesis) and therapeutic interventions (vesicoamniotic shunts). When ultrasound has proved unsatisfactory (e.g., in cases of maternal obesity or anhydramnios), magnetic resonance imaging is superior to provide sufficient anatomical detail.

Table 4 Society of fetal urology grading system of antenatal hydronephrosis

Grade	Renal pelvic complex	Renal parenchymal thickness
0	Intact	Normal
I	Mild splitting	Normal
II	Moderate splitting (confined to renal border)	Normal
III	Marked splitting (outside renal border, calyceal dilatation)	Normal
IV	Pelvicalyceal dilatation	Thin

Whereas fetal upper urinary tract obstruction, particularly unilateral, portends a more favorable prognosis, fetal lower urinary tract obstruction often has a less favorable outcome, due to associated cystic renal dysplasia, oligohydramnios, pulmonary hypoplasia, dysmorphia, and limb contractures. Prenatal diagnosis allows for the planning of appropriate prenatal and postnatal care, consideration of prenatal intervention, and psychological adjustment of the parents. For some fetal abnormalities of the lower urinary tract, uncertainty exists regarding best management [80]. The use of fetal urine samples, fetal serum, amniotic fluid, and/or fetal renal biopsies is still not widely used clinically. Also, the prognostic value of markers to assess fetal renal function is uncertain (with few exceptions) and the effectiveness of therapy remains to be established [80, 81]. The main goal of performing imaging studies early is to distinguish those kidneys that do not require surgery from those that will deteriorate and lose function or growth potential and thus benefit from surgery.

Fetal Urine Analysis

In the presence of severe fetal obstructive uropathy (e.g., posterior urethral valves) the analysis of fetal urine is most commonly used before performing *in utero* therapy. The first urine sample may not reflect kidney function, as this urine may have been in the bladder for some time. Sequential sampling of fetal urine seems to improve the accuracy. Often, values that remain stable or even increase over time are suggestive of renal dysplasia. Electrolytes (sodium, calcium, and chloride), α_1-microglobulin, β_2-microglobulin, total protein, microalbumin, N-acetyl-β-glucosaminidase, osmolality, and other urinary analytes (IGF-1, IGF-BP3, creatinine, and cystatin C) have been used as biomarkers for fetal kidney function. Recently, fetal urinary peptides have been shown to predict postnatal renal function in fetuses with posterior urethral valves [81]. Among more than 4000 fetal urinary peptides, 26 peptides were associated with end-stage kidney disease before the age of 2 years [81]. Fetal tubular dysfunction is suggested by increased urinary sodium concentrations. At 20–30 weeks of gestation, sodium concentrations in the fetal urine normally fall below 90 mmol/l. Fetal urinary sodium or chloride concentrations above 100 mmol/l have been shown to be predictive of fetal or perinatal death from renal and/or pulmonary failure [82]. Values of fetal urinary β_2-microglobulin > 13 mg/l, which is normally filtered by the glomerulus and $> 99.9\%$ reabsorbed in the proximal tubule, are associated with perinatal demise. The two tests most widely used to determine the need for *in utero* surgery were urinary calcium and sodium concentrations >95th percentile for gestation; however, a recent systematic review to evaluate the usefulness of fetal urine analysis in congenital urinary tract obstruction concluded that the evidence to support its use is insufficient [83].

Postnatal Diagnosis

In upper urinary tract obstruction (e.g., ureteropelvic junction obstruction) patients who elude antenatal diagnosis, may develop abdominal pain or discomfort, hematuria, and/or urinary tract infections. Recurrent episodes of vomiting may not be recognized to be related to obstructive uropathy. Patients with congenital anomalies (VATER/VACTER-L association, congenital heart disease, and CNS malformations) may be found to have urinary tract obstruction during the workup for their defects.

In patients with lower urinary tract obstruction, the presence of abdominal masses (bladder, kidney), delayed voiding (e.g., urethral valves), deficient abdominal wall musculature, or undescended testes (e.g., Eagle-Barratt syndrome) indicate the need for further evaluation. Unfortunately, a normal voiding pattern does not exclude urinary tract obstruction. In infants with a palpable bladder and in female newborns with interlabial cystic masses (prolapsed ureterocele) bladder outlet obstruction should be considered. Delayed diagnosis of lower urinary tract obstruction in children may lead to chronic kidney disease, hypertension, recurrent urinary tract infections, sepsis, growth failure, and urinary incontinence.

Imaging and Nuclear Medicine Studies

The evaluation of congenital obstructive uropathy includes the assessment of morphologic and structural changes (e.g., dilatation of the urinary tract, bladder wall thickness, kidney parenchyma) as well as the assessment of functional changes (e.g., impaired ^{99m}Tc-MAG-3 excretion or differential renal uptake). A combination of repeated imaging and nuclear scan examinations provides complementary information and helps to establish the diagnosis (see chapter “Diagnostic Imaging”).

Ultrasonography

Ultrasonography is the first noninvasive diagnostic test in urinary tract obstruction pre- and postnatally. It is widely available, relatively inexpensive, and allows for the morphological assessment of both the lower and upper urinary tract. Ultrasonography can be difficult to perform in patients who are not cooperative. Assessment of the fetal urinary tract is part of all routine prenatal ultrasound examinations. Dilatation of the renal pelvis and fluid filled areas as small as 1–2 mm may be visualized *in utero* using high-resolution real-time ultrasound. Most clinically relevant urinary tract obstructions are currently detected in the second trimester. A fetal pelvic dilatation of > 3 mm during the second trimester and > 6 mm after 32 weeks of gestation is considered abnormal. Postnatal evaluation is recommended in all cases of prenatal hydronephrosis. In congenital obstructive uropathy, a renal ultrasound should be performed within the first week of life and repeated 4–6 weeks later. The first postnatal ultrasound should not be performed within the first few days of life, when the kidneys produce little urine. This can mask hydronephrosis and give rise to false-negative results. After the first week postdelivery, a renal ultrasound should be performed in term infants with both a full and an empty bladder; the sonographer should assess renal pelvic dilatation, calyceal dilatation, pelvic or ureteral wall thickening, and signs of renal dysplasia. In the absence of calyceal dilatation, the degree of obstruction is minimal. In these patients functional imaging such as a nuclear renal scan is not recommended. In contrast, dilatation of all renal calyces suggests a greater degree of obstruction, even with less renal pelvic dilatation. The renal pelvis diameter should be measured in the transverse plane and at the anteroposterior diameter. An anteroposterior diameter (APD) of >7 mm in a newborn is considered abnormal (in an older child >1 cm is abnormal). All measurements should be adjusted to age and size and compared with normal values. Pelvic dilatation is often used as an indicator of severity, yet may be overestimated with an extra-renal pelvis, which is clinically irrelevant. It is important to examine the contralateral normal kidney for hypertrophy, which is an important indicator for decreased function in the obstructed kidney.

The combination of APD and diffuse calyceal dilatation at first postnatal ultrasound can distinguish patients with a moderate degree of hydronephrosis who have a higher risk for requiring surgery to relieve urinary tract obstruction. A study of 348 children with antenatal hydronephrosis demonstrated that the predictive capability of this combination was 14-fold better compared to APD alone for patients with APD at 9–15 mm or greater [84]. The absolute percentage difference of ipsilateral and contralateral renal lengths appears to be an additional predictor of the need for pyeloplasty in UPJO [85]. A prospective study of 213 infants found that postnatal ultrasound testing as 5 days and 1 month after birth had high sensitivity and negative predictive value for significant urological disease. Moreover, if two sequential postnatal ultrasound tests showed normal kidneys and urinary tract, only 3% of patients had will have any pathology, which was usually low-grade vesicoureteral reflux [86]. In infants with postnatal APD $\leq$7 mm and no calyceal, kidney, ureteral, or bladder pathology, there is no need for long-term follow-up [87]. If necessary, further investigations include a voiding cystourethrogram, nuclear scan, and (rarely) magnetic resonance imaging to detect and determine degree of reflux and/or obstruction.

Voiding Cystourethrogram

The voiding cystourethrogram (VCUG) is an essential part of most basic evaluations of patients with hydronephrosis. The VCUG provides anatomical information in terms of bladder morphology, bladder capacity, patency of the urethra, bladder emptying, and in case of reflux an anatomical description of the upper urinary tract. It still serves as the gold standard for detecting posterior urethral valves, vesicoureteral reflux, ureteroceles, and bladder diverticulae (Fig. 1). Vesicoureteral reflux can both cause and coexist with hydronephrosis; it can contribute to additional obstruction due to ureteral dilatation and should be assessed early. Grading of reflux can be difficult if obstruction is present. Because of

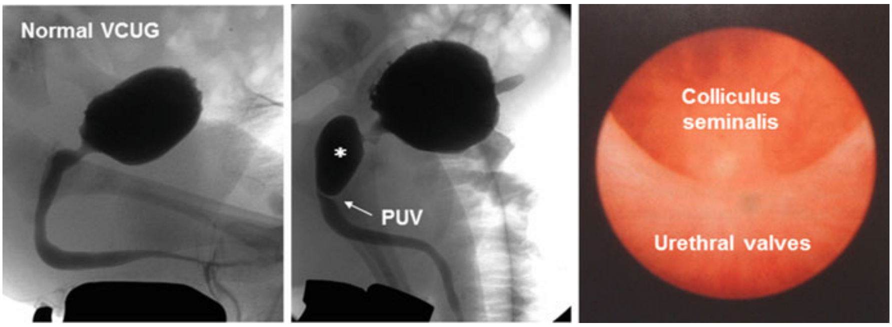


Fig. 1 Posterior urethral valves (PUV) on a voiding cysturethrogram with dilated posterior urethra (*). Endoscopic image of PUV (Courtesy Dr. B. Kammer, Dept. Radiology and Dr. H. G. Dietz, Dept. Surgery, Dr. V. Hauner Children's Hospital, LMU, Munich)

the relatively high incidence of reflux in children with congenital urinary tract obstruction, many physicians keep patients on antibiotic prophylaxis until the VCUG is obtained.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a noninvasive, radiation-free imaging modality, which is considered ideal for assessment of detailed anatomy, particularly of ectopic ureteral insertion, complicated duplex systems, or when other complex pathologies are suspected. The administration of a specific non-nephrotoxic intravenous contrast medium allows for the functional evaluation of parenchymal perfusion, glomerular filtration, and renal excretory function. Indications for MRI include preoperative anatomic imaging, and the assessment of vascular anatomy. With its fine resolution, the presence of a crossing blood vessel causing obstruction may be detected. The MRI may also differentiate between hydronephrosis and parapelvic cysts. It provides excellent anatomic and functional details of the collecting system. Thus, ureteral strictures in children can be diagnosed more accurately [88]. Its use is restricted in younger children by the need for sedation, its high costs, and limited availability. The correlation with functional outcomes in obstructive uropathy has yet to be established.

Diuretic Renography

Nuclear medicine studies play an important role in the functional assessment of the kidneys. Radionuclide scintigraphy can evaluate relative renal function, estimate absolute renal function, differentiate between functional renal parenchyma and renal scars, estimate renal blood flow, and measure excretory function [89, 90]. Several radiopharmaceuticals are available to image the kidney and monitor function. ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA), ^{99m}Tc-dimercaptosuccinic acid (DMSA), and ^{99m}Tc-mercaptoacetyltriglycine (MAG-3) are the most commonly used radionuclides. In children with obstructive uropathies, ^{99m}Tc-MAG-3 is the main radiopharmaceutical for renal imaging. Following intravenous administration ^{99m}Tc-MAG-3 is highly protein-bound and removed from the plasma primarily by the organic anion transporter 1 located on the basolateral membrane of the proximal renal tubules in the kidney. ^{99m}Tc-MAG-3 accumulates in proximal tubular cells and is secreted into the tubular lumen via organic anion transporters on the apical membrane. If the apical transporter is impaired to a greater extent than the basolateral transporter, ^{99m}Tc-MAG-3 activity is retained in the kidneys. In general, the amount of tracer uptake in the first 2 min after injection correlates with the glomerular filtration rate. The ^{99m}Tc-MAG-3 scintigraphy sometimes described as tubular extraction rate can be used as a measure of relative renal function. Relative renal function is indicated as the relative percentage of the right and the left kidney function. While each kidney normally contributes 50%, due to error, relative renal function is not typically considered to be abnormal until it is < 40%. Decreased renal function either reflects unilateral renal impairment or congenital anomalies (e.g., dysplasia). Normal relative function results may be also obtained with bilaterally damaged kidneys. Because of its efficient extraction (more than twice that of ^{99m}Tc-DTPA), ^{99m}Tc-MAG-3 is preferred in patients with suspected obstruction and impaired kidney function. Multiple protocols have been used for ^{99m}Tc-MAG-3 nuclear scans with different time points of furosemide administration and variable acquisition times [89, 90]. The "F=0 protocol" is the most convenient protocol, in which furosemide (1mg/kg with a maximum dose of 20 mg in a child) is administered simultaneously with the tracer. The "F+20 protocol" involves giving furosemide 20 min after administering the radionuclide that then allows for the direct evaluation of the tracer-washout. The washout phase is used to examine the severity of obstruction; it is conventionally assessed by measuring the half-time (T1/2) after furosemide administration.

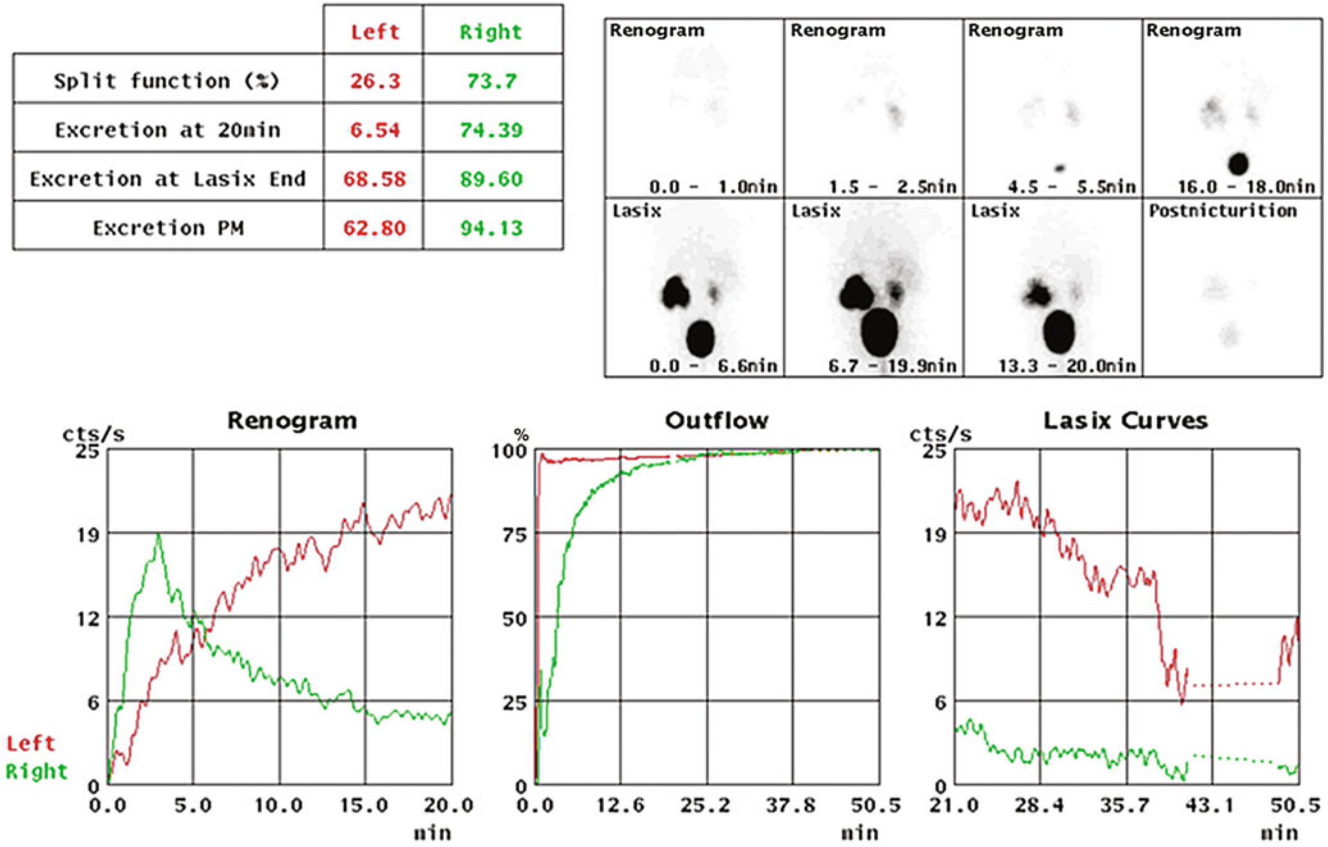
The nuclear scan report describes a ^{99m}Tc-MAG-3-time-activity-curve for each individual kidney. The normal curve shows a steep rise, followed by an early peak and a rapidly declining phase. In contrast, urinary tract obstruction with dilatation results in a continuously rising curve. The application of furosemide is used to differentiate between obstruction and "reservoir effect." The time required to clear one-half of ^{99m}Tc-MAG-3 is monitored in minutes (T1/2). A half-time <10 min is considered normal, 10–20 min is uncertain, and >20 min is consistent with obstruction (Fig. 2). That said, a prolonged T1/2 should never be the sole criterion for diagnosing obstruction. Clinical correlation with ultrasound is mandatory and sequential nuclear scans may also be helpful. The Santa Fe Consensus report recommends a 35-min acquisition with furosemide administered 20 min after the tracer. This diuretic MAG3 renography with the "F+20 protocol" is the method of choice in young children with obstructive uropathy. In addition, criteria have been developed for children that allow the furosemide administration to be omitted when the baseline drainage is normal [91]. ^{99m}Tc-MAG-3 scintigraphy can be used as early as at the end of the first week of life to assess differential renal function. Because immature nephrons of the newborn kidney are less sensitive to furosemide, a diuretic ^{99m}Tc-MAG-3 study should be postponed until the patient is at least 4–6 weeks old to allow for nephron maturation. In the typical patient with unilateral hydronephrosis and dilated calyces, ^{99m}Tc-MAG3-scintigraphy would be performed at 2–3 months of age and repeated 3–12 months later depending on the initial results obtained (see Fig. 3 with proposed algorithm for therapeutic approach to postnatal asymptomatic hydronephrosis, adapted from [92]). Patterns of change over time are probably the most useful markers to indicate the status of the affected kidney.

Other tracers, outside of ^{99m}Tc-MAG-3 can also be used

^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA) is the only renal radiopharmaceutical available for routine imaging that is purely filtered by the glomerulus. Consequently, it can be used to measure glomerular filtration rate. ^{99m}Tc-dimercaptosuccinic acid (DMSA) is an excellent cortical imaging agent that is used primarily to evaluate relative function, pyelonephritis, and renal scarring [93]. Approximately 40% of the injected dose is retained by the renal tubules within 1 h after injection. ^{99m}Tc-DMSA initially binds to α_1-microglobulin; the complex is then filtered by the glomerulus and accumulates in the kidney via megalin/tubulin-mediated endocytosis in the proximal tubule [94]. Thus, the renal uptake of ^{99m}Tc-DMSA depends on renal blood flow, glomerular filtration, and proximal tubule receptor-mediated endocytosis. In summary, nuclear scans remain the "gold-standard" assays for the functional assessments of the kidney with obstruction, although better diagnostic tools are clearly needed.

Excretory Urography and Pressure Perfusion Studies

The intravenous pyelogram (IVP) has practically vanished, except for rare circumstances and conditions such as urolithiasis or pre- and postoperative evaluation in complex conditions, particularly when other imaging tools are not available or applicable. In selected cases, in which the anatomy is not



	Left	Right
Split function (%)	26.3	73.7
Excretion at 20min	6.54	74.39
Excretion at Lasix End	68.58	89.60
Excretion PM	62.80	94.13

Renogram
0.0 - 1.0min
Renogram
1.5 - 2.5min
Renogram
4.5 - 5.5min
Renogram
16.0 - 18.0min
Lasix
0.0 - 6.6min
Lasix
6.7 - 19.9min
Lasix
13.3 - 20.0min
Postnicturition
Renogram
cts/s
25
19
12
6
0
0.0
5.0
10.0
15.0
20.0
min
Left
Right
Outflow
%
100
75
50
25
0
0.0
12.6
25.2
37.8
50.5
min
Lasix Curves
cts/s
25
19
12
6
0
21.0
28.4
35.7
43.1
50.5
min

Fig. 2 MAG-3 diuretic renogram in ureteropelvic junction obstruction (UPJO), demonstrating reduced split function and delayed washout of the tracer on the left side, suspicious for obstruction. (Courtesy Dr. T. Pfluger, Dept. Nuclear Medicine, LMU, Munich)

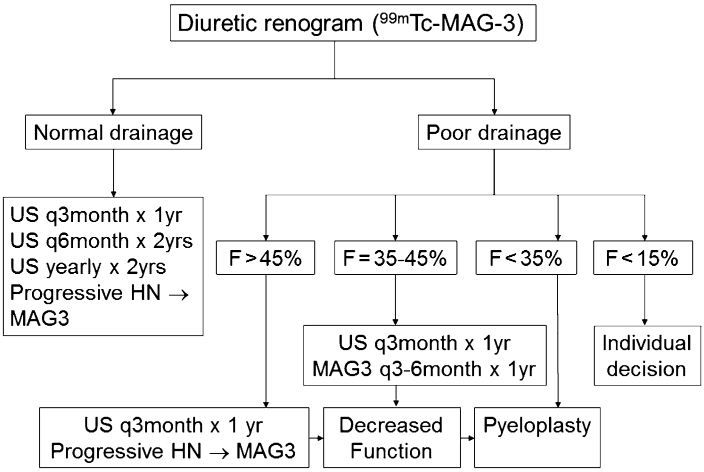


Fig. 3 Proposed algorithm for the evaluation of UPJO with hydronephrosis grade 3–4. *US* ultrasound, *HN* hydronephrosis, *F* split function. (Adapted from [92])

satisfactorily defined, the IVP can provide structural and functional information of the urinary tract. The "Whitaker test," which was developed in the late 1960s and early 1970s, combines an urodynamic study with antegrade pyelography to assess potential upper urinary tract obstruction. The test involves instilling contrast directly into the affected kidney and requires general anesthesia. The "Whitaker test" may differentiate between patients with residual or recurrent obstruction and those with dilatation secondary to nonobstructing permanent ureteral changes. In selected cases it can be used to determine when percutaneous nephrostomy tubes can be safely removed in postoperative patients.

Ureteropelvic Junction Obstruction

Ureteropelvic junction obstruction (UPJO) is the most common cause of prenatal obstructive hydronephrosis and has an incidence of 1 in 1000–1500 newborns. UPJO is more common on the left side (2:1 – left to right) and more common in boys (2:1 – male to female). Bilateral obstruction is rare. UPJO is characterized by an obstruction at the proximal portion of the ureter where the pelvis narrows into the ureter. The obstruction can be classified as intramural, mural, or extramural. Intramural causes are exceptionally rare in children and include abnormal mucosal valves or fibroepithelial polyps. Mural obstructions, which are most common, result from a dysfunctional or adynamic ureteral segment that has an abnormal distribution of smooth muscle cells and collagen fibers. The main proposed mechanisms for the development of mural UPJO are failure in the development of the renal pelvis, abnormal pyeloureteral innervation, and impaired smooth muscle cell differentiation [95]. In mural UPJO the lumen is almost always patent, albeit narrow and irregular. Extramural or extrinsic causes, which are less common, include aberrant or crossing renal vessels, artery and/or vein, bands or adhesions. Usually a lower pole renal vessel fixes the ureter in position; however, not all "crossing vessels" are obstructive. These vessels may also cause intermittent reductions in urine flow leading to intermittent dilatation of the pelvis. In the majority of UPJO the underlying cause is thought to be functional (i.e., mural).

Development of the Ureter and the Ureteral Peristaltic Machinery

The kidney starts to produce urine at ~10 weeks of gestation. At the same time, the trunk of the ureteric bud starts elongating without branching to form the early ureter. Similar to the kidney, the morphogenesis of the ureter requires a close interaction between the inner ureteral epithelial cells and the surrounding ureteral mesenchymal cells. In response to the molecular signals from the epithelial and mesenchymal cells, the early simple cuboidal ureteral epithelial cells differentiate into the multilayered urothelium (also called transitional epithelium). The urothelial surface is covered by urothelial plaques expressing uroplakin proteins and is impermeable to urine [46]. Meanwhile, the mesenchymal cells differentiate into stromal cells, smooth muscle cells, and adventitial fibroblasts (eventually becoming the serosa). Differentiated smooth muscle cells are organized into layers with inner circular and outer longitudinal orientations and express α-smooth muscle actin [49, 54]. Interestingly, ureter smooth muscle differentiation occurs in an ascending fashion from the distal ureter close to the bladder to the proximal ureter that is attached to the renal pelvis. Peristaltic waves are initiated in the renal pelvis and are propagated through the ureter wall to the bladder. Periodic coordinated sequential contractions of the pelvis and ureter are controlled by two distinct populations of urinary tract pacemaker cells (PMCs). PMCs at the pelvic-kidney junction are characterized by expression of hyperpolarization-activated cyclic nucleotide-gated channel 3 (HCN3) and low-voltage-gated T-type calcium channel TTC (also termed CaV 3.1) [57, 96]. Urinary tract PMCs located adjacent to the smooth muscle layer in the proximal ureter are termed Interstitial Cells of Cajal-like cells (ICC-LCs) and express cKIT. The ICC-LCs are located sparsely throughout the ureter (including renal pelvis, UPJ, UVJ) [97]. However, the distribution of ICC-LCs is not even, with most ICC-LCs located in the proximal renal pelvis and reduced cell density in the distal segments of the ureter. The ICC-LCs can produce electrical slow-wave potentials that control the propagation of the peristaltic activity. The molecular mechanism and physiological functions of the ureteral peristaltic machinery are still unclear. It has been suggested that HCN3-expressing PMCs in the tip of the renal pelvis act as the primary pacemaker to initiate spontaneous potentials that are propagated and modulated by ICC-LCs to the adjacent typical SMCs in order to trigger intermediate action potentials and ureteral smooth muscle contraction. The ICC-LCs may also produce additional autorhythmicity and can take over pacemaking in the renal pelvis and ureter in the absence of the primary pacemaker activity [97]. Both populations of urinary pacemaker cells are derived from neural crest and can be detected as early as mouse E12.5 in the tissues surrounding the embryonic kidney [98]. Mouse Kit-expressing ICC-LCs are first detected at E15.5 in a subset of renal epithelial cells and cells of the ureteropelvic adventitia. This developmental time point is followed by the beginning of urine production by the mouse embryonic kidney around E16.5 and is well before the initial ureteral peristaltic waves, which are first observed around E18.5 in mice [98]. It is still unclear when the ICC-LCs appear in the human fetal ureter and at what embryonic stage human fetuses start to have rhythmic ureteral peristalsis.

Genetics of Ureteropelvic Junction Obstruction (UPJO)

Weisschuh et al. have described a ureteropelvic junction obstruction phenotype in patients with Axenfeld-Rieger syndrome (Table 2) [99]. The Axenfeld-Rieger syndrome is caused by mutations in FOXC1, a transcription factor of the forkhead family that is highly expressed in the metanephric mesenchyme. In addition, mutations in the transcription factor *FOXF1*, another member of the forkhead box genes, cause the “Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins” (ACDMPV) syndrome. ACDMPV is associated with ureteral valve-like constriction at the ureteropelvic junction, tortuous ureters, and hydronephrosis [99, 100]. *FOXF1* is located at the same chromosomal locus as *FOXC2*, and is a part of the sonic hedgehog and TGF-β signaling pathway (Table 2) [101]. Other genes in the sonic hedgehog and TGF-β signaling pathways play critical roles in lower urinary tract development and congenital anomalies. Analysis of obstructing ureteric tissue in infants and children with congenital UPJO demonstrated upregulation of hedgehog signaling effectors and stromal genes suggesting that increased hedgehog signaling may contribute to the pathogenesis of human UPJO [102]. Single gene mutations in genes including TBX18, ROBO2, FREM2, HPSE2, AGTR2, BMP4, and ID2 are proposed in the etiopathogenesis of UPJO [34, 95].

Clinical Presentation in UPJO

Prior to using fetal ultrasound, patients with UPJO were identified due to their signs and symptoms. Patients were commonly diagnosed between the ages of 6 and 15 years, with only 14% being identified younger than 1 year of age [103]. Today, most UPJO is detected prenatally, but some may still present later in life with acute flank pain, nausea, vomiting, and abdominal discomfort. Many of these patients are misdiagnosed as having primary gastrointestinal disease. The pain usually results from intermittent obstruction of the urinary tract when the urine flow overwhelms the capacity to drain. The pattern of abrupt onset with severe abdominal pain, nausea, and vomiting, often in the late evening is typical for older children with UPJO. Late-onset hypertension can occur in patients with obstruction [104]. Urinary tract infection (UTI) is an uncommon presentation in UPJO, but if present, may be quite severe requiring urgent intervention and drainage. Vesicoureteral reflux (VUR) should be evaluated in patients with UPJO and UTI, since the incidence of VUR in infants and young children who present with pyelonephritis is higher than any other anomaly of the urinary tract including obstruction. Fortunately, most children with UPJO are asymptomatic and rarely require intervention.

Management of UPJO

The majority of patients with UPJO can be managed conservatively with close observation and serial imaging [92, 105]. This is usually accomplished by regular repetitive ultrasound testing in well-hydrated infants and children using standardized ultrasound grading and pelvicalyceal system measurements, complemented by intermittent ^{99m}Tc-MAG-3 diuretic scans, if the ultrasound demonstrates suspicion of deterioration. The postnatal evaluation of a child with prenatally detected hydronephrosis should begin with an ultrasound, usually between 48 and 72 h after birth, due to transient neonatal dehydration (which can mask hydronephrosis). However, ultrasound testing is recommended earlier in specific cases, such as bilateral hydronephrosis, solitary kidney or a history of oligohydramnios. Spontaneous resolution of hydronephrosis within the first weeks of life has been shown for 17% of cases [106]. Of the remaining infants, 72%, 19%, and 2% demonstrate mild (5–9 mm), moderate (10–15 mm), and severe (> 15 mm) dilatation, respectively [106]. Whereas almost 90% of cases with mild or moderate hydronephrosis resolve or stabilize within the first 2 years of life, the remaining 10% show persistent moderate to severe dilatation. Approximately 25% of infants with severe postnatal dilatation on initial ultrasound will stabilize or resolve to normal or mild dilation, while 50% stabilize with moderate dilatation within the first 2 years. The remaining 25% of initially severely dilated cases will show persistent severe dilatation [78, 106]. Predicting a future need for surgical pyeloplasty in patients with moderate to severe pelvic dilatation is challenging. As discussed above, the combination of APD and diffuse calyceal dilatation at first postnatal ultrasound can predict, as much as 14-fold better than APD alone, those with a moderate degree of hydronephrosis who have a higher risk for requiring surgery to relieve urinary tract obstruction [84].

Overall, in infants with moderately severe to severe hydronephrosis with caliectasis will need ongoing monitoring and functional assessment. This should include a ^{99m}Tc-MAG-3 diuretic renogram to evaluate differential function and wash-out times (Fig. 2). If the initial function is above 45% in patients with a poor drainage pattern, ultrasound evaluation should be repeated at 3 and 12 months. If the initial differential function is between 35% and 45%, follow-up ^{99m}Tc-MAG-3 may be obtained in 3 months. Ultrasound evaluation should be repeated every 3 months. Progressive hydronephrosis, decreasing differential renal function, recurrent urinary tract infections, and/or urolithiasis may suggest the need for surgical intervention (Fig. 3 with proposed algorithm for therapeutic approach to postnatal asymptomatic hydronephrosis grade 3–4). If the initial relative function is below 35%, surgery to relieve obstruction should be considered. Most surgical interventions for UPJO are performed within the first 2 years of life [107]. In the very small group of newborns with severe UPJO, early surgical correction is safe and efficient and should not be postponed [108]. According to a recent meta-analysis including more than 1000 patients, approximately four out of five children with unilateral UPJO may be treated safely with nonsurgical management. However, approximately 25% of all conservatively followed patients that ultimately required surgical intervention later required a second surgical intervention [105]. High-grade hydronephrosis in neonates is unlikely to resolve spontaneously and is often accompanied by a loss of renal function [109]. For children with less severe hydronephrosis, functional imaging is not usually useful unless regular ultrasound detects progressive hydronephrosis with diffuse calyceal dilatation [109]. Ultrasound intervals are usually increased over time, although most children with UPJO are followed until normal. Closer monitoring should be exercised in patients with a higher risk for adverse outcome (bilateral hydronephrosis, solitary kidney with UPJO).

At present, indications for surgical correction of UPJO vary between institutions, countries, and continents. In a retrospective study of 343 children with prenatally diagnosed UPJO that were initially conservatively managed, over 50% eventually required surgical treatment [107]. Advanced skill and experience as well as new surgical techniques have improved surgical results even in very early infancy (e.g., neonatal pyeloplasty, endoscopic, and/or endoluminal pyeloplasty) [92, 108]. Laparoscopic pyeloplasty has been used for over two decades with good results and low morbidity, but still has limited availability in many centers. With the advent of robotic surgical technology, the learning curve for this type of laparoscopic surgery is markedly shortened [110]. The largest to date series on robotic pyeloplasty in infants includes 62 surgeries in 60 patients with a mean age of 7.3 months and a median weight of 8.1 kg. Resolution or improvement of hydronephrosis was documented in 91% of kidneys [110].

The open Anderson-Hynes dismembered pyeloplasty is still considered the gold standard for relief of UPJO. This procedure can be performed in the flank position or through a dorsal lumbar incision, both of which are cosmetically reasonable. The procedure includes the resection of the stenotic segment, some size reduction of the dilated pelvis, and re-anastomosis of the ureter to the renal pelvis. Success rates are in the 95–97% range. Following UPJO surgery, recovery is quick in infants but slower in older children. Percutaneous nephrostomy (PCN) was more regularly performed one or two decades ago, particularly in neonates with “gross dilatation” as “bridging” until surgery. Today PCN is typically restricted to untreatable pyonephrosis, severe bilateral obstruction with renal insufficiency, or in a severely obstructed single kidney with elevated serum creatinine.

Biomarkers in UPJO

The lack of precise and reliable biomarkers for severity of urinary tract obstruction is problematic [111]. Numerous urinary biomarkers (TGF-β, TNF-α, N-acetyl-β-D-glucosaminidase, Epidermal Growth factor (EGF), CCL2, angiotensinogen, Prostaglandin reductase 1 (PTGR1), Ficolin-2, Nicotinate-nucleotide pyrophosphorylase, Immunoglobulin superfamily containing leucine-rich repeat protein (ISLR), vascular cell adhesion molecule-1 (VCAM-1), metalloproteinases, antimicrobial peptides, and polyomavirus) and serum metabolomics have been evaluated in patients with UPJO [112–122]. The detection of urinary polypeptides by capillary electrophoresis and mass spectrometry (CE-MS) was shown to help predict the outcome of infants with UPJO [123, 124]. A recent meta-analysis included 14 studies with reported data on 380 UPJO patients who underwent surgery, 174 who were treated conservatively, and 213 controls. The authors concluded that some biomarkers offer promising results, but acknowledge that more multicenter, prospective studies are needed to evaluate their diagnostic and prognostic value [125].

Long-Term Follow-Up in UPJO

The long-term outcome is usually positive for most patients with UPJO. Nevertheless, long-term studies have been sparse. Many children undergoing successful pyeloplasty are lost for follow-up after several years. In light of the experimental evidence, as well as the limited clinical data, it is very likely that some of these patients will develop proteinuria, hypertension, or chronic renal insufficiency. In one long-term follow-up study of children after pyeloplasty for UPJO, renal function measured by nuclide scan improved in all but one patient, but was persistently abnormal in 14 of 33 patients [126]. Renal function improved significantly in children undergoing surgery at <1 year of age, but normalized in only one of six patients >1 year of age [126]. Children undergoing pyeloplasty for grade IV hydronephrosis showed a broad range of renal interstitial fibrosis [114]. Delay of pyeloplasty in these children was associated with greater macrophage infiltration, and a lower glomerular filtration rate [114]. In addition, renal interstitial fibrosis can be found in kidney biopsies of UPJO patients and correlates significantly with the differential function of the affected kidney [27]. Differences in the urinary peptidome excretion at 5-year follow-up may indicate permanent changes in the kidney or urinary tract in nonoperated patients with UPJO, while based on clinical parameters alone these children appear to have normal kidneys [127]. These data strongly support the need for long-term follow-up, even in less severe cases of UPJO. For the long-term prognosis it will be important to determine whether these changes are deleterious or the result of an ongoing and possibly beneficial remodeling process.

Ureterovesical Junction Obstruction

Ureterovesical junction obstruction (UVJO) is characterized by an obstruction at the distal end of the ureter where it meets the bladder and results in a megaureter. The term obstructive megaureter or primary obstructive megaureter (POM) is often used for this entity, and should be distinguished from a refluxing megaureter. UVJO is usually caused by an adynamic segment of the distal ureter. Similar to UPJO, histological studies show changes of disordered smooth muscle cells and abnormal collagen deposition. UVJO is less common than UPJO and is reportedly responsible for 9–14%of the cases of hydronephrosis.. UJVO is seen more often in boys and is usually unilateral with more left-sided involvement.

Pathogenesis of UVJO

The "bud theory" developed by Mackie and Stevens explains that the ureteral orifice derives from the original ureteric budding site on the Wolffian duct during embryologic development [128]. When the ureteric buds arise at abnormal sites of the Wolffian duct, the final sites of the ureteric orifices may be also abnormal, resulting in disturbed ureterovesical junctions, vesicoureteral reflux, or UJVO. In addition, multiple ureteric buds may also lead to short duplex ureters, duplex kidneys, and ectopic and dysplastic kidneys [24, 43, 128, 129]. Therefore, mutations of genes controlling early ureteric bud formation and positioning can cause ureteral phenotypes such like urinary obstruction, vesicoureteral reflux, and hydronephrosis [129, 130]. Additionally, maldevelopment of the ureter and its peristaltic machinery may cause UVJO as well (see section "Development of the Ureter and the Ureteral Peristaltic Machinery").

Clinical Presentation in UVJO

Most UVJO is detected prenatally. Urinary tract infection is a frequent clinical presentation of UVJO and should prompt evaluation for additional vesicoureteral reflux in infants and young children. Many physicians keep patients with UVJO on antibiotic prophylaxis during the first 6–12 months. During acute infection, the megaureter may appear far more dilated than before, which is presumably due to the effect of bacterial toxins on the ureteral muscle tone. Abdominal or flank pain, nausea, and vomiting are less common in UVJO.

Diagnosis and Management of UVJO

Diagnosis of UVJO is usually made by ultrasound. Only rarely may ureteral valves be present that can pose a problem, as they can easily be missed. As with UPJO, most cases of UVJO resolve spontaneously. The pattern of hydroureteronephrosis resolution is cranial to caudal and often the distal dilatation is the last to disappear, which can take years. Isolated distal dilatation as a residue of UVJO may be found in older children and adults. While UJVO was initially often operated early, there is now general agreement that a conservative approach is usually feasible without endangering the kidney. With the conservative approach repeated follow-up ultrasound studies are essential. Monitoring for urinary tract infection, often with uncommon bacteria or atypical pathogens such as fungus, is mandatory. Peristalsis of the obstructive megaureter can be assessed sonographically when using cine loop clips or M-mode tracks and the ureteral inflow jet. For quantification of drainage, a diuretic ^{99m}Tc-MAG-3 scintigraphy with an “F+20 protocol” should be performed with good hydration as well as bladder emptying and upright positioning for assuring maximum drainage. Approximately 10% of UVJO patients will need surgical correction. It is important to differentiate those children who do well with primary reimplantation from those who benefit from temporary diversion by ureterocutaneostomy to enable maturation and retonisation of the ureter.

Megacystis Microcolon Intestinal Hypoperistalsis Syndrome

Megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) is an extremely rare cause of antenatally detected urinary tract dilatation. MMIHS is a cloacal plate anomaly that is more often seen in females. It is a rare genetic disease with autosomal-recessive inheritance. MMIHS is characterized by a dilated bladder and hydronephrosis in the presence of normal or increased amniotic fluid. Smooth muscle cells in the bladder and distal bowel are dysfunctional. Tissue analysis of patients with MMIHS demonstrates a lack of the alpha$_3$-nicotinic acetylcholine receptor subunit giving a possible explanation for the underlying pathogenesis in some cases.

Prune Belly Syndrome (Eagle-Barratt Syndrome)

Prune belly syndrome (PBS) is a rare lower urinary tract malformation affecting about 1 in 30,000 newborns [131]. PBS consists of a triad of typical features, which include a partial or complete lack of abdominal wall musculature with a dry prune like wrinkly skin appearance of the abdomen (hence the name “prune belly”), the dilation of the proximal and distal urinary tract with hydronephrosis, hydroureters, and a distended thin-walled bladder, and bilateral cryptorchidism. All these signs are present in the neonatal period [132]. The majority of PBS patients are males. Coexisting morbidities include pulmonary hypoplasia, vesicoureteral reflux, abnormalities of the urethra, dysplastic kidneys, which commonly lead to chronic renal failure and kidney transplantation. Mutations in *HNF-1B* and *CHRM3* gene have been described in patients with PBS [133, 134]. The prenatal presentation is similar to posterior urethral valves (PUV). In contrast to PUV, both sexes can be affected in PBS, and the so-called “keyhole sign,” which is typical for PUV, is missing on ultrasound.

Posterior Urethral Valves (PUV)

Posterior urethral valves (PUV) are the most common cause of lower urinary tract obstruction in male infants. They are characterized by the formation of sail-like membrane folds from the verumontanum to the prostatic urethra, which causes severe bladder outlet obstruction exclusively in boys. PUV occur in 1:5000–8000 live births and are observed in 0.003% of fetal ultrasound screenings [135]. PUV are the most common cause of obstructive uropathy leading to end-stage renal failure and as such has high rates of morbidity and mortality [3].

Development of the Bladder and Urethra

Unlike the mesodermal ontogenetic origin of the kidney and ureter, the bladder and urethra arise from the endodermal urogenital sinus after the urorectal septum (i.e., Tourneux's fold) separates the embryonic cloaca into the ventral urogenital sinus and the dorsal rectum. At around 5 weeks of human gestation, the urogenital sinus is further separated into the anterior vesicourethral canal and the posterior urogenital sinus. The anterior portion of the urogenital sinus becomes the bladder, which has an open outflow tract at its apex that is connected to the allantois during early fetal development. This outflow tract is only functional at the early embryonic stage to drain the developing bladder to the allantois through the umbilical cord. By ~15 weeks of human gestation, the bladder separates from the umbilicus that becomes a remnant called the urachus, which is further stretched to become the median umbilical ligament. Concurrently, the posterior vesicourethral canal becomes the pelvic portion of urethra in the male (which has three segments: pre-prostatic, prostatic, and membranous segments) and the entire urethra in the female [136]. The mechanisms by which PUV form is still unclear [137].

Pathophysiology of PUV

The classic form of PUV is found in the prostatic urethra, below or proximal to the verumontanum. Although the precise mechanism of PUV formation is unknown, four theories have been proposed to explain their development. They include hypertrophy of the urethral mucosal folds, persistence and continuation of the urogenital membrane, abnormal development of the Wolffian or Mullerian duct, and fusion of the verumontanum or the posterior urethral roof epithelium [138]. The upstream effects of PUV may be profound, leading to abnormal renal development in the most severe cases. PUV may result in massive hydronephrosis and ureteral dilatation; the bladder can be markedly thickened with muscle hypertrophy and matrix deposition. Severe obstructions are associated with oligohydramnios and pulmonary hypoplasia, which causes high mortality and morbidity.

Genetics of PUV

Recently it could be shown that rare variants in BNC2, encoding basonuclin are implicated in familial autosomal-dominant PUV and cause anatomical blockage of the bladder outflow [139].

Clinical Presentation and Diagnosis

Most patients with PUV are detected prenatally by ultrasonography. The typical findings at second trimester ultrasound are bilateral hydronephrosis, distended bladder, dilated posterior urethra (keyhole sign), and thickened bladder wall in a male fetus. Increased echogenicity and cysts in the fetal kidney by ultrasound testing are suggestive of renal dysplasia. Amniotic fluid volume, associated anomalies, and fetal sex need to be assessed by ultrasound (for prenatal diagnosis see section "Prenatal Diagnosis"). Unless there is consideration for antenatal intervention, there is little change in obstetric management. Early delivery is not beneficial and exposes the child to the risks of prematurity. There are no contraindications to vaginal delivery in PUV. Postnatally, the definitive diagnosis of PUV can be made by VCUG. Older children with PUV that were missed on prenatal ultrasound (or that did not have a prenatal ultrasound test) may present with urinary tract infections, urosepsis, renal failure, or urinary incontinence. In most cases there will be evidence of an abnormality on ultrasound, which can be then confirmed by VCUG.

Management of PUV

The clinical management of PUV continues to challenge pediatric nephrologists and urologists due to the wide spectrum of severity, the variable clinical progression, the inability to predict long-term consequences, and the inability to alter renal damage once it is established.

Prenatal Management of PUV

Early intrauterine shunting (vesicoamniotic or vesicoperitoneal shunt) does not improve outcome and carries the risk of severe complications. Vesicoamniotic shunting was first reported as a potential therapy in 1982. Since then, a few case series have suggested that survival could be improved in fetuses that undergo this procedure [140]. Recently, however, in a randomized trial in the UK, Ireland, and the Netherlands, women whose pregnancies with a male fetus were complicated by PUV were randomly assigned to receive either the intervention (placement of vesicoamniotic shunt) or conservative management [80]. The primary outcome was survival of the patient to 28 days postnatally. Survival was slightly higher in the fetuses receiving vesicoamniotic shunting, but the size and direction of the effect remained uncertain, such that benefit could not be conclusively proven. The results showed that the chance of newborn babies surviving with normal renal function is very low irrespective of whether or not vesicoamniotic shunting is done [80]. Unfortunately, the trial was stopped because of poor recruitment. At present, this prenatal surgical procedure is recommended only in fetuses with relatively unimpaired fetal urine samples (see section "Fetal Urine Analysis"). If untreated, fetal lower urinary tract obstruction carries a mortality rate of 45%, which is mostly from associated severe oligohydramnios in the mid-trimester with pulmonary hypoplasia. The initial management of infants with lower urinary tract obstruction may therefore require respiratory support and intensive care.

Postnatal Management of PUV

In suspected posterior urethral valves early postnatal ultrasound is performed. Perineal ultrasound may suggest valves, but a VCUG is still mandatory. Usually early intervention is necessary to ensure sufficient urinary drainage by catheterization, cystostomy, or percutaneous nephrostomy before transurethral valve resection can be performed. The postnatal management focuses on preventing further renal damage that could arise by urinary tract infections. The commonly observed renal damage in these neonates is usually an irreversible renal dysplasia. Adverse prognostic factors in children with PUV include the gestational age at diagnosis, the presence of VUR, echogenic kidneys at the time of diagnosis, the serum creatinine at 1 year of age (>1 mg/dl), the presence of urinary tract infections, and proteinuria and hypertension later in life. Consistent with these factors, a retrospective analysis of 685 children with PUV diagnosed at a median age of 7 days demonstrated that coincident renal dysplasia carries a 9-fold risk of kidney transplant [141]. Long-term urethral obstruction is potentially associated with cystic renal dysplasia and abnormal renal function (glomerular and tubular). Of those who survive the neonatal period, 25–30% develop end-stage renal disease necessitating dialysis and/or transplantation. Optimal management of the lower urinary tract is essential for preserving renal function. Bladder dysfunction requires close monitoring and regular urodynamic investigations [142]. In some patients, decreased renal concentrating ability will expose the bladder to high urine volumes, which will further aggravate a small capacity, stiff and poorly compliant bladder. Recognition and interruption of this cycle of voiding dysfunction is important. Urinary tract infections in children with PUV carry the risk of further deterioration of kidney function and should be treated promptly.

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Bladder Disorders

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Tryggve Nevéus, Carlos R. Estrada, and Paul F. Austin

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Abstract

Lower urinary tract (LUT) dysfunction is clinically relevant for three main reasons: (1) it may cause urinary tract infections (UTIs), mainly from the accumulation of residual urine; (2) it may adversely affect renal function from febrile UTIs or high intravesical pressure; and (3) it may cause incontinence and thus substantially lower the child's quality of life.

The function of the LUT is intimately associated with that of the bowel; constipation is both a common cause and consequence of many kinds of bladder dysfunction. Among the types of lower urinary tract dysfunction, *detrusor overactivity* is the main cause of daytime urinary incontinence in childhood. The condition is benign but socially harmful and needs to be differentiated from more serious conditions such as neurogenic bladder or urethral obstruction. The two cornerstones of treatment are urotherapy and eradication of concomitant constipation. Urotherapy includes instructing the child to keep a regular voiding and drinking schedule. If first-line therapy is unsuccessful, anticholinergic medication is often warranted. Another LUT disorder, *voiding dysfunction*, i.e., the habitual sphincter contraction during voiding, is common in children with detrusor overactivity. It may be self-limiting but may also result in residual urine and UTIs and can in severe cases be part of a syndrome indistinguishable from the neurogenic bladder and carry the same risks. A third disorder, *neurogenic bladder dysfunction*, most often caused by spinal dysraphism, is characterized by detrusor sphincter dyssynergia. This condition very often leads to recurrent UTIs and high intravesical pressures, threatening kidney function. The introduction of clean intermittent catheterization has improved the renal prognosis tremendously for the children affected by the disorder.

Keywords

Incontinence · Voiding dysfunction · Neurogenic bladder · Urotherapy · Urodynamics

Background

Definitions and Terminology

Throughout this text, the globally accepted lower urinary tract (LUT) terminology, as outlined by the International Children's Continence Society (ICCS), will be strictly adhered to [1]. The specific terms that appear italicized are defined below:

- *Incontinence* means any involuntary leakage of urine and is divided into *continuous* and *intermittent* varieties, the former denoting the situation when urine is constantly leaking and the latter when leakage comes in discrete portions. The word *intermittent* may be omitted when it is clear from the context that the *continuous* variant is not being referred to.
- *Enuresis* means intermittent incontinence at night while asleep (see Chap. 60 on enuresis).
- *Voiding dysfunction* (or dysfunctional voiding) is the term used when urine flow during voiding is diminished by sphincter contractions. Please note that this term should not be used as an umbrella term for all types of LUT dysfunction.
- The child with *overactive bladder* experiences urgency symptoms (i.e., sudden, unexpected urge to void), with or without incontinence, which is then called *urge incontinence*. The term *detrusor overactivity* is warranted when uninhibited detrusor contractions have been documented via cystometry.
- *Holding maneuvers* are behaviors such as standing on tiptoe or squatting with the heel pressed against the perineum or digital

compression on the genitalia, used by children as an often unconscious response to detrusor contractions. *Voiding postponement* is the term used when these maneuvers are used habitually.

- *Underactive bladder* means that the child needs to strain to void. When insufficient or absent detrusor contractions have been detected cystometrically, the term *detrusor underactivity* may be used.
- When the storage function of the bladder has been assessed using voiding charts, *voided volumes* have been measured. The terms *bladder volume* or *capacity* may only be used when the amount of urine actually inside the bladder has been measured, via ultrasound or catheterization. However, when a standard for comparison is needed, the *expected bladder capacity* (EBC) can be calculated using the formula EBC $= 30 \times (\text{age} + 1)$ mL, which is valid until the adult EBC of 4 dL is reached at age 12 [2].

Tools of Investigation

The use of *voiding charts* is crucial in the evaluation of LUT function in children after they no longer require diapers [1]. A standard voiding chart is created by having the child void in a measuring cup with each micturition for 2 days, and the symptoms such as urgency, enuresis, and daytime incontinence are documented for one or 2 weeks. Ideally, bowel movements and stool consistency (e.g., Bristol Stool Scale) should also be included. Crucial data that can be extracted from the voiding chart include micturition frequency, maximum and average voided volumes (preferably expressed as a percentage of EBC for the child's age), and symptom frequency. The use of voiding charts is strongly recommended in the evaluation of any child with incontinence or other LUT complaints as this provides objective data for analysis.

For children old enough to void on command, the use of *flowmetry* is beneficial, especially for those with suspected voiding problems (e.g., weak stream or the need to strain to void) or with recurrent urinary tract infections (UTIs) secondary to incomplete bladder emptying or therapy-resistant incontinence. To calculate flowmetry, a child will void on a toilet that measures the flow rate, and immediately afterward, the amount of residual urine still in the bladder is assessed via an ultrasound device. The following uroflow curve types are associated with specific voiding dynamics (see Fig. 1): (1) the bell-shaped curve which is normal, (2) the tower-shaped curve which is either normal or indicative of detrusor overactivity, (3) the staccato curve which indicates dysfunctional voiding, (4) the plateau curve which raises the suspicion of anatomical outflow obstruction, and (5) the intermittent curve which is typical of detrusor underactivity. Residual urine volume in excess of 20 mL is pathological. Please note, however, that a pathological curve needs to be verified by successive flowmetry studies before even tentative conclusions may be drawn.

When a noninvasive screening of the LUT function of infants and children before the age of bladder control is needed, a *4-h voiding observation* is the method of choice [3], if feasible. For this test, the child is allowed to play and eat freely for 4 h while every void is documented by weighing diapers, and ultrasound is used to assessment residual urine volume. If bladder function is normal, there should be at least one complete void without residual urine during the observation period.

Invasive *cystometric evaluation* of LUT function is mainly indicated when the history, physical examination, and/or noninvasive investigation suggest the possibility of neurogenic bladder. Urodynamic evaluation is typically performed by simultaneously measuring bladder pressure via a transurethral pressure-transducing catheter, intra-abdominal pressure via a transrectal probe, and sphincter activity via perineal patch electrodes. The bladder is filled at a slow rate during which pressure is monitored continuously. Variables including presence of overactive contractions, bladder filling pressures, leak point pressure, voiding pressure, post-void residual volume, and relaxation of the sphincter muscle are recorded. These data allow comprehensive evaluation of LUT function.

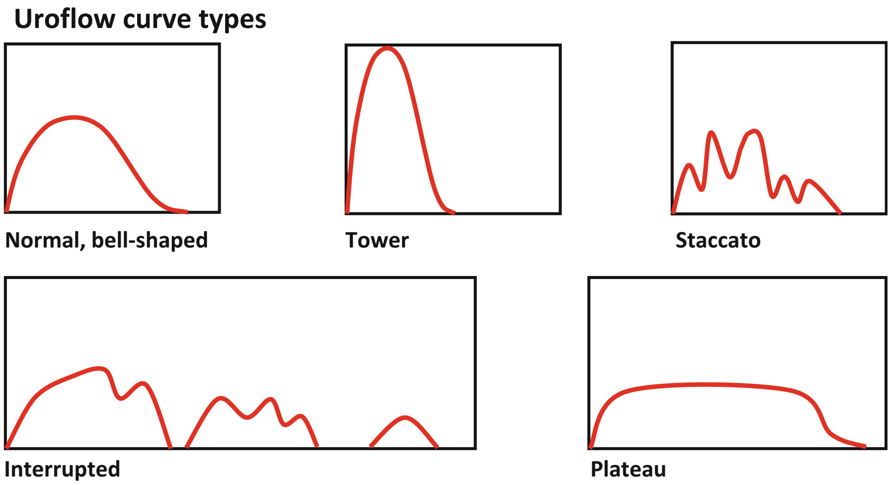

Fig. 1 Uroflow curves

If one suspects constipation, especially if history is ambiguous and rectal examination is not feasible, ultrasonographical measurement of the horizontal rectal diameter behind the bladder can be used. If this diameter is above 30–35 mm in a child without the urge to defecate, then constipation is highly likely [4, 5]. In addition, the Bristol Stool Scale can be used to assess the consistency of stool.

Development of LUT Function

All the neural circuits and pathways for bladder control are present in utero during which urine is voided in small, discrete amounts. The infant is, of course, not socially continent, and bladder emptying is “reflexive”; however, detrusor overactivity is rare, and voiding is usually accompanied by cortical arousal. At the infant stage of development, LUT immaturity with detrusor-sphincter dyscoordination is normal, resulting in interrupted, incomplete voids; however, the bladder is usually completely emptied at least once per 4 h.

The first step toward voluntary bladder control, usually occurring in the second year of life, happens when the child is able to sense and communicate that voiding has occurred or is happening. This phase coincides with bladder enlargement and neural maturation. Next, the child becomes able to postpone micturition for a short while, remaining continent provided a toilet is readily available and an adult is there to help. Subsequently, the child develops the ability to start micturition even when the bladder is not full and no urge to void is sensed. The final stage of bladder control includes the ability to interrupt an ongoing voiding. Complete social continence is achieved by approximately 90% of children by the age of 4–5 years [6]. Children can become continent much earlier by gentle but persistent encouragement of timed voids; [7] this strategy may be recommended for small children with recurrent UTIs. Normal voiding frequency is 5–8 voids per day.

Bladder-Bowel Interaction

It has become increasingly clear that LUT function is very much influenced by the bowel and vice versa. In many children with predominantly urological problems, an underlying bowel issue, e.g., constipation, can be shown to be the main causative factor [8, 9]. There are several potential mechanisms that may explain this link. First, the bowel and the LUT share a large part of their neuromuscular innervation [10]. Second, fecal impaction means that the rectum – which should normally be a signal space, not a storage organ – is fairly constantly dilated and compresses the bladder from behind [11]. Third, behavioral issues such as voiding postponement may not only influence the bladder but also bowel habits [12]. Thus, constipation may be a crucial contributing factor in urinary stasis with recurrent UTIs, detrusor overactivity (with or without incontinence), urinary retention, and voiding dysfunction – including the extreme form severe bladder-bowel dysfunction (see below). Finally, children with neurogenic bladder almost always also suffer from neurogenic bowel disturbance.

According to the Rome IV definition [13, 14], at least two of the following criteria should be present for at least 4 weeks to meet the definition of constipation: (1) two or fewer bowel movements per week, (2) a history of excessive stool retention, (3) painful/hard bowel movements, (4) large-diameter stools that may obstruct the toilet, (5) fecal incontinence once per week or more, and/or (6) the presence of a large fecal mass in the rectum as assessed by ultrasound or rectal palpation.

Conditions

Detrusor Overactivity

Idiopathic detrusor overactivity, often associated with a positive family history, is the most common cause of daytime incontinence. Although there is a clear link to constipation, as mentioned above, the etiology is unknown. Previous notions about urinary incontinence being primarily a psychiatric disorder appear to be incorrect. However, children with daytime incontinence are, for unclear reasons, overrepresented among those with neuropsychiatric disorders such as attention deficit hyperactivity disorder (ADHD); furthermore, incontinence per se may cause low self-esteem and thus secondary psychosocial problems [15].

Invariably, these children experience urgency symptoms, but the problem that makes the family seek medical attention is usually incontinence. Concomitant enuresis (see Chap. 60) is common, as is constipation and/or fecal incontinence. The urinary incontinence typically varies from small wet spots to complete bladder emptying, with and without urgency; furthermore, voiding charts often reveal that these children urinate at the toilet (even ≥ 8 times per day). Holding maneuvers are common. Fortunately, the condition is benign, and most children will have resolution with older age; however, if the child is old enough to be bothered by the incontinence (usually by age six or older), they should be treated (see below).

Dysfunctional Voiding

Dysfunctional voiding (as defined above) may be a learned behavior as a way for the child with detrusor overactivity to manage the involuntary detrusor contractions. In other cases, the pathogenesis is less clear. Importantly, it may also be the first sign of an undetected neurogenic bladder disturbance or be a component of severe bladder-bowel dysfunction (see below) [16, 17]. Patients with dysfunctional voiding will have uroflow studies that consistently show staccato curves. Residual urine is also usually present. As the condition can only be diagnosed by flowmetry, it is unclear how common it is in asymptomatic children. It is usually discovered when investigating a child with recurrent UTIs or therapy-resistant urge incontinence. Patients with this condition are also typically constipated [17]. While dysfunctional voiding may be benign and remit spontaneously, it may also lead to recurrent UTIs secondary to incomplete bladder emptying and urinary stasis and in the worst cases lead to upper tract renal damage. In a child with UTIs and/or hydronephrosis, dysfunctional voiding should be aggressively treated.

Severe Bladder-Bowel Dysfunction

This condition has previously been known as the Hinman-Allen syndrome or non-neurogenic neurogenic bladder [18]. Patients with this condition have voiding dysfunction combined with elevated detrusor filling and/or voiding pressures that are seen in neurogenic bladders, without discernible damage to the spinal cord. Severe BBD may result in an end-stage bladder disease with urinary retention. These patients almost always also have severe constipation, always have high residual urine volumes, and may have upper tract disease. The etiology is largely unknown and probably heterogeneous [17]. In some cases, it may represent the extreme end of the spectrum of learned dysfunctional behavior, starting with ordinary detrusor overactivity, proceeding to dysfunctional voiding resulting in irreversible bladder damage with detrusor underactivity. Rarely, children may have severe BBD from Ochoa syndrome, a recessively inherited condition, characterized by urological dysfunction and the inability to smile [19]. Unfortunately, severe bladder-bowel dysfunction has an unfavorable natural history. It will not remit spontaneously, will often cause recurrent UTIs, and may even lead to end-stage renal disease if left untreated [20].

Neurogenic Bladder

Any damage to the spinal cord, congenital or acquired, can result in neurogenic bladder (and bowel) dysfunction. In children, the most common cause by far is myelomeningocele (MMC). Also, spinal cysts and lipomas that result in spinal cord tethering may be causative. Other causes include malformations such as sacral agenesis or VACTERL syndrome. The LUT of the child with spinal cord damage usually manifests in detrusor sphincter dyssynergia (DSD), i.e., the detrusor and sphincter contractions are not coordinated, which results in high-pressure contractions and incomplete emptying. These problems result in pathological remodeling of the detrusor muscle, which can ultimately lead to poor bladder dynamics that place the kidneys at risk [21]. Although most cases of neurogenic bladder are recognized at birth, some may be detected when evaluating children with recurrent UTIs or therapy-resistant incontinence with noninvasive urodynamics and/or imaging studies. Physical signs that should raise the suspicion of occult spinal dysraphism are lumbar hair tufts or dimples, asymmetrical gluteal cleft, different length of the lower extremities, and/or foot deformities [22].

Although renal function is usually normal at birth, recurrent febrile UTIs and/or high intravesical pressure can often lead to progressive renal failure in the long term, if the LUT (and the bowel) is not treated. Furthermore, many of these children have difficulties reaching continence without expert help. As the child with MMC grows, there is a risk of the spinal cord becoming tethered to the spinal canal, resulting in vertebral deformity (scoliosis), lower extremity deformity, gait, and/or LUT function. A tethered spinal cord should be suspected whenever there is unexplained deterioration of LUT function [23].

Special Conditions

Extraordinary daytime urinary frequency syndrome (EDUFS) is diagnosed in children, usually toddlers, who need to urinate at the toilet very often (more than once per hour) but whose nocturnal bladder function is completely normal for their age. The pathogenesis of the condition is unknown, but the prognosis is excellent [24].

Vaginal reflux, i.e., the entrapment of a moderate amount of urine in the vagina during micturition, may affect prepubertal girls and results in incontinence that consistently occurs shortly after normal voiding. The problem will usually spontaneously disappear by puberty [25].

Giggle incontinence is a rare condition which may be overdiagnosed but also frequently missed. The etiology of this condition is unknown. These patients are typically teenage girls and exhibit urinary incontinence only when laughing. Otherwise, LUT function is completely normal. The condition should be carefully differentiated from both detrusor overactivity, when leakage occurs extra easily when the child's focus is lapsing, and stress urinary incontinence when leakage occurs with pelvic floor laxity during increased intra-abdominal pressure. The leakage in giggle incontinence is linked to laughing only. This condition usually will not remit spontaneously and can be debilitating [26].

Evaluation

The Incontinent Child

Most children with (intermittent) daytime urinary incontinence have overactive bladder. The purpose of the initial evaluation is to differentiate

Table 1 Warning signs in the incontinent child

Signs/symptoms	Possible explanation	Action
Continuous incontinence	Ureteral ectopy	Consult urologist
Poor stream, need to strain to void	Obstruction. Neurogenic bladder. Voiding dysfunction	Noninvasive urodynamics
Stress incontinence	Neurogenic bladder	Noninvasive urodynamics, consult urologist
General symptoms: nausea, weight loss, polydipsia, poor growth	Chronic kidney disease	Urine dipstick, fluid measurements, creatinine, ultrasound
Recurrent UTIs	Residual urine	Noninvasive urodynamics
Lower back findings, leg/buttock asymmetry	Spinal dysraphism	Invasive urodynamics, consider MRI

overactive bladder from other causes, which may lead to different types of treatment signs (see Table 1). The history should focus on the type of incontinence, bladder and bowel habits, development, the presence of general symptoms, and whether the patient has had UTIs. The physical examination should include inspection of the genitals and a neurological examination of the lower limb. The physician should look for signs of spinal dysraphism (see above). Rectal examination is valuable, if feasible, since the presence of formed feces in the *ampulla recti* without a present urge to defecate is virtually pathognomonic for constipation.

The *voiding chart* (as described above) is an essential part of the evaluation of the incontinent child. In addition to providing important LUT data, it can be used to assess the family's adherence and will engage the child and thus form part of therapy.

A *urine dipstick test* is recommended during the initial evaluation of all incontinent children. If leukocyturia is observed, a urine culture is warranted. Please note, however, that asymptomatic bacteriuria – a benign condition that should not prompt antibiotic therapy – is common in children with LUT dysfunction [27, 28]. Thus, in a child with incontinence that is not of recent onset and is not accompanied by dysuria, UTI is less likely a cause for the incontinence, even in the presence of leukocyturia and bacteriuria. However, acute cystitis can cause incontinence from bladder irritability with OAB. Finally, the presence of glucose on urinalysis of a patient with incontinence should prompt evaluation for diabetes mellitus without delay.

Noninvasive urodynamic evaluation is not needed for the initial evaluation unless there are warning signs such as poor stream, need to strain to void, or recurrent UTIs with pyelonephritis. In addition therapy-resistant children should have noninvasive urodynamic evaluation. *Invasive* urodynamics are only indicated if there is a suspicion of neurogenic bladder or if the noninvasive evaluation is consistently pathological.

Daytime incontinence per se is usually not an indication for imaging studies. One would likely perform ultrasound testing of the kidney and upper urinary tracts in patients with general symptoms (nausea, weight loss, polydipsia, poor growth) or a history of febrile UTIs. Ultrasound of the rectal diameter can be useful if there is doubt whether constipation is present or not.

The Child with Abnormal Voiding Frequency, Voiding Difficulties or Urine Retention

A patient with *frequent voiding* often has urgency and as such fulfills the criteria for overactive bladder and should be evaluated in the same way as the incontinent child. A voiding chart including 2 days of urine measurements is important since a minority of these children void often because they have pathological polyuria due to a renal concentrating defect. A urine dipstick test is needed if symptoms started recently or if dysuria is present. Children with EDUFS (see above) require no specific evaluation other than possibly an ultrasound of the bladder to rule out extrinsic bladder compression from fecal impaction or a tumor. A bladder ultrasound test is also warranted to rule out impaction or tumor in children whose symptoms persist or progress for several months.

The patient with *infrequent voiding* is often diagnosed in a child beyond diaper age who has had UTIs. If average voided volumes are significantly elevated (above 130% of EBC for the child's age) or if UTIs are frequent, then noninvasive urodynamic investigation should be initiated. As always, concomitant constipation or bowel dysfunction should be ruled out.

The child with a weak or *poor urinary stream* and/or *urinary straining to void* should be evaluated with noninvasive urodynamics to rule out neurogenic bladder or severe bladder-bowel dysfunction that may pose risks for upper urinary tract and kidney damage. Additionally, these children may have an unrecognized urethral stricture or posterior urethral valves. A screening ultrasound of their kidneys and LUT is useful to evaluate for the presence of hydroureteronephrosis and/or bladder wall thickening. A retrograde urethrogram, voiding cystourethrography (VCUG), or cystoscopy can be selectively used to evaluate patients in whom one suspects bladder outlet obstruction.

The majority of children with *urinary retention* have acquired this as a postoperative complication or are constipated. If the patient has not had surgery and/or constipation is not immediately apparent or if the retention is persistent or recurrent, then ultrasound of the LUT should be performed to rule out fecal impaction or a tumor. A urologist should be consulted, and invasive urodynamics may be indicated.

Other Children Needing Evaluation of Bladder Function

Since most UTIs in children after the age of 2–3 years are caused by LUT dysfunction with incomplete emptying, many children with recurrent UTIs need evaluation of LUT and bowel function. Most recommend that noninvasive urodynamic evaluation (uroflow and residual urine volume) is done in all boys with more than one UTI and all girls with 3–4 or more UTIs. Boys should be evaluated with fewer UTIs since ascending bacteriuria is less common in males and often associated with other congenital anomalies, such as previously undetected posterior urethral valves.

Infants with recurrent UTIs that have associated vesicoureteral reflux (VUR) should undergo 4-h voiding observation (if feasible), since these infants are often not able to completely empty their bladders [29]. *Severe VUR*, i.e., grade IV or V, may be primary and anatomical but may also be secondary to LUT dysfunction. It is advisable to address any LUT dysfunction before correcting the VUR (see Chap. 57 on vesicoureteral reflux). Thus, most recommend that noninvasive urodynamic investigation (4-h voiding observation or uroflow and residual, according to age) be performed in children with high-grade reflux to aid in the management strategy.

Children with evidence of poor renal function of unclear etiology who are found to have hydronephrosis should be urodynamically evaluated, since the poor renal function may be the result of long-standing severe bladder-bowel dysfunction or an occult neurogenic bladder. Noninvasive evaluation of LUT function is required in all children before *renal transplantation*, since untreated poor bladder function may be deleterious for graft function and longevity.

Children with known *spinal dysraphism* need invasive cystometric evaluation in the neonatal period and usually a few additional times later in life. More intensive evaluation is needed when spinal cord tethering is suspected (see above). Most often, patients with suspected occult spinal dysraphism require invasive urodynamics.

Treatment

Children who are distressed by their LUT dysfunction, at risk for recurrent UTIs, and/or at risk for damage to the upper urinary tracts and kidneys should be treated. In most patients, therapy is reserved for those with bothersome symptoms. Those patients that have abnormal voiding chart data or that urinate frequently or seldom with mild urgency or occasional wet spot but are not bothered by these issues need no treatment.

Overactive Bladder

The mainstay of treatment of the child with urge incontinence (urgency being the hallmark of OAB) consists of two strategies: eradication of constipation, if present, and basic bladder advice/urotherapy [30]. Additionally, in the setting of complete bladder emptying, OAB may also be treated with bladder relaxants or antimuscarinics in addition to basic urotherapy. These drugs should not be used when emptying is incomplete.

Treatment of the frequently associated constipation can significantly improve the course of patients with OAB [31]. The child needs to unlearn a maladaptive stool behavior, thus leading to shrinkage of the distended rectum and return of the rectum to function as a signal space, not a storage organ. These behavioral therapies take time, effort, and patience. One frequently used initial strategy is to start with daily mini-enemas (if these are tolerated) for several days, followed by polyethylene glycol daily, with dosage adjusted so that the child has daily soft bowel movements without fecal incontinence. Concurrently, the child is encouraged to keep physically active, hydrate well, consume a healthy diet, and, importantly, never postpone bowel movements. The therapy should continue for a minimum of 3 months [31].

Basic urotherapy includes ensuring that the child orally hydrates well and urinates at the toilet approximately every 2 h (while awake), regardless of feeling the need to void. Voiding body posture should be reviewed with the family including that the child should relax, have support for the feet and thighs, and not rush through visits at the toilet. Voiding schedules and voiding diaries are essential tools for the behavioral-oriented urotherapy. The use of a watch with a vibrating alarm function set for every 2 h is also beneficial, especially when the parents are less available for supervision [32]. Basic urotherapy is usually feasible from age 6 years or older and should be followed for 2–3 months before evaluating treatment success. Approximately half of children treated with basic urotherapy will have resolution of their incontinence [33]; many of the nonresponders appear to have poor outcomes simply because of inadequate adherence to the voiding schedule (and/or bowel treatment). Finally, some children may need the help of a child psychologist or psychiatrist if they have concomitant neuropsychiatric issues.

As noted, OAB with complete emptying or OAB patients who have failed standard urotherapy may require bladder relaxants such as anticholinergic pharmacotherapy to assist with their LUT dysfunction. Many drugs are available, although only oxybutynin has been approved for use in children. Alternative drugs including fesoterodine, tolterodine, and solifenacin appear to have better side-effect profiles and as such are on track for pediatric labeling [34–36]. Before anticholinergic therapy is started, however, the practitioner needs to address several issues: (1) the patient needs to be evaluated for constipation and, if present, treated; (2) significant residual urine needs to be assessed for and addressed if noted; (3) regular micturition habits need to be maintained; and (4) good oral hygiene needs to be ensured. The most common side effect of anticholinergic therapy in childhood is constipation, but there are also risks for incomplete voiding leading to residual urine and, with either constipation or high residual urine volumes, risks for UTIs. Oxybutynin and its metabolites also carry a risk for central nervous system side effects (mood swings, aggression), and most drugs will also diminish saliva production [34]. A typical dosage of oxybutynin is 2.5–5 mg two or three times daily. The effect of therapy is evaluated after 1–2 months. If patients have an initial good response that later diminishes, then constipation should be suspected and treated. If patients develop UTIs during therapy, the drug should be discontinued, and bladder emptying should be evaluated with measurement of residual urine volumes. Likewise, if treatment is needed for long term, then residual urine volumes should be checked every 6 months. Drug tapering is recommended, typically four times per year, to check whether pharmacological treatment is still needed.

Other interventions, outside of anticholinergic drugs, are potentially available to patients. The sympathomimetic drug mirabegron has shown low toxicity and good efficacy as an additional therapy in adults who are nonresponders to anticholinergic monotherapy [37]. More research is needed to determine whether this drug can be used for pediatric overactive bladder [38]. Finally, transcutaneous electrical stimulation, invasive sacral nerve stimulation, and intradetrusor botulinum toxin injections have been used as third-line therapies in cases of severe treatment resistance.

Dysfunctional Voiding

Treatment of dysfunctional voiding is indicated in patients with UTIs or upper tract damage. Care should be taken to exclude neurogenic bladder or severe bladder-bowel dysfunction. After constipation has been ruled out or treated, the first-line therapy is urotherapy with biofeedback [39]. This requires an experienced therapist and collaboration with the child who as a result of training should learn how to distinguish between a coordinated voiding and one with concomitant sphincter contraction. If this therapy is unsuccessful, then alpha-blocker medication may be considered. Severely therapy-resistant voiding dysfunction in a child at risk for UTIs, renal damage, or intractable incontinence should be treated with clean intermittent catheterization (CIC) as in patients with neurogenic bladders.

Neurogenic Bladder or Severe Bladder-Bowel Dysfunction

The treatment of the neurogenic bladder or severe bladder-bowel dysfunction demands the close collaboration between a skilled urotherapist and physicians with pediatric and urological expertise [40]. The cornerstone of treatment is CIC, which should preferably be managed by the child as soon as he/she has attained sufficient maturity. The purpose of therapy is threefold: (1) protection of renal function, (2) prevention of UTIs, and (3) attainment of social continence. For the growing child, the latter of these goals is usually the most important. Almost all children with these conditions will also need aggressive bowel management. If CIC and bowel management alone do not suffice, various additional therapies may be needed, such as intravesical anticholinergics, alpha-blockers, intradetrusor botulinum toxin, or, as a last resort, surgical bladder augmentation and reconstruction of the bladder outlet. Spinal cord tethering will need to be treated neurosurgically [23].

Special Conditions

There are no proven therapies for EDUFS, although anticholinergics, nonsteroidal anti-inflammatory drugs, and behavior therapy have been attempted. Fortunately, most of these children are preschool age, and the prognosis is excellent, regardless of any attempted interventions [41].

Girls with vaginal reflux should be advised to void with their legs held wide apart; one means of achieving this if they are using a western-style toilet is to have the child face the back of the toilet when voiding. If this maneuver does not produce the desired effects, application of a piece of toilet tissue to the perineum with gentle manual pressure after each void usually leads to extrusion of the residual urine [25].

Giggle incontinence is difficult to treat, and as such, patients need to be managed by practitioners with sufficient expertise. Advanced urotherapy (e.g., biofeedback) or off-label use of central nervous system stimulant medication (e.g., methylphenidate) appears to offer the best chances of success [42].

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Enuresis

59

Søren Rittig

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Abstract

Nocturnal enuresis or bedwetting is among the most prevalent chronic diseases in childhood and also represents one of the most feared conditions among children and adolescents

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due to the associated myths and negative stigmatization. The aim of this chapter is to provide an updated review of current knowledge on nocturnal enuresis spanning from definitions and epidemiology through new scientific studies on pathogenesis and genetics to internationally accepted protocols for evaluation and treatment. Wherever possible, reference will be made to evidence-based findings and recommendations bearing in mind that low level of evidence is not uncommon in this pediatric field. This chapter complements the data presented in the chapter on bladder disorders (Chap. 58, "Bladder Disorders").

Keywords

Nocturnal enuresis · Children · Pathogenesis · Evaluation · Treatment

Introduction

Nocturnal enuresis or bedwetting is among the most prevalent chronic diseases in childhood and also represents one of the most feared conditions among children and adolescents due to the associated myths and negative stigmatization. The aim of this chapter is to provide an updated review of current knowledge on nocturnal enuresis spanning from definitions and epidemiology through new scientific studies on pathogenesis and genetics to internationally accepted protocols for evaluation and treatment. Wherever possible, reference will be made to evidence-based findings and recommendations' bearing in mind that low level of evidence is not uncommon in this pediatric field.

Definitions

Enuresis Subtypes

Nocturnal enuresis (NE) is defined by the International Children's Continence Society as urinary incontinence during night while asleep in child aged 5 years or more and with a frequency of at least one episode per month [1, 2]. Although no uniform guidelines are used as to the frequency, many divide NE into frequent (≥4 per week) of infrequent (<4 per week) enuresis and into primary and secondary NE dependent on whether the child has experienced a symptom-free period of at least 6 months [2]. Also, NE is classified into *monosymptomatic* NE (MNE) if the child has no associated lower urinary tract (LUT) symptoms and as *non-monosymptomatic* NE (NMNE) if the child has LUT symptoms such as daytime urinary incontinence, urgency, voiding difficulties (such as poor stream, hesitancy, straining), or abnormal voiding pattern (low or high). The background behind this latter distinction is the fact that bladder dysfunction (such as overactive bladder) is more common in children with NMNE with direct implications on management.

Reduced Bladder Capacity

Reduced storage capacity of the bladder is an important aspect of NE pathogenesis (see below) as well as management and is substantiated by recording voided volumes on a frequency volume chart during 2 days. The observed maximal voided volume (MVV) is compared with age-expected bladder capacity (EBC) (valid until age 12 or max. 400 ml) using the formula:

$$\text{EBC} = (\text{age in years}) \times 30 + 30\ \text{mL}.$$

Reduced MVV is defined as MVV <65% of EBC.

Nocturnal Polyuria

Nocturnal urine production is also a crucial variable in both NE pathogenesis and management and this can be estimated by 1 week of home recording based upon diaper weights and first morning voided volume (nocturnal urine volume = difference in diaper weight before and after sleep plus first morning void). Nocturnal polyuria is defined pragmatically by the International Children's Continence Society (ICCS) as a nocturnal urine volume on a night with enuresis exceeding 130% of EBC of the child. An alternative population-based definition defines age-related polyuria as nocturnal

Table 1 The table shows the age-related reference values for maximal voided volumes (MVV) estimated by frequency-volume charts and for nocturnal urine volume estimated by home recordings (see section "Diaries")

Age	Normal bladder capacity (MVV)	Reduced bladder capacity (MVV)	Nocturnal polyuria on wet night
5 years	180 ml	<117 ml	>234 ml
6 years	210 ml	<136 ml	>273 ml
7 years	240 ml	<156 ml	>312 ml
8 years	270 ml	<175 ml	>351 ml
9 years	300 ml	<195 ml	>390 ml
10 years	330 ml	<214 ml	>429 ml

urine volume exceeding 20 × ((age in years) + 9) ml [3] (Table 1).

Epidemiology

Prevalence

Nocturnal enuresis is worldwide among the most common chronic diseases in childhood and more common in boys than girls (M/F ratio of approximately 1.4) [4], especially in the younger age groups below 12 years [4–6]. The exact prevalence is variably described from 4–24% dependent primarily on different diagnostic criteria used regarding frequency of NE [7]. Thus, prevalence rates are considerably lower in children with more severe enuresis, for example, twice or more episodes per week [8]. Most studies report prevalence in 7-year-olds of 5–10% [8–11] declining to approx. 1–2% in young adults [4]. Although early data indicated a spontaneous resolution rate of approx. 15% annually [12], more recent studies have clearly shown that children with frequent NE have more severe NE [4, 8] and a much lower resolution rate. The exact distribution between enuresis subtypes is not clearly established but one study found that NMNE is more common (approximately 75%) than MNE [13].

Comorbidities and Consequences

Constipation, Fecal Incontinence, and Urinary Tract Infection (BBD)

Although NE is commonly seen alone (MNE) it can be associated with other elimination disorders such as constipation, fecal incontinence and urinary tract infection (UTI), an entity known as *bladder and bowel dysfunction* (BBD)(ICCS). The proportion of NE patients with BBD symptoms is not clear but these patients are important to identify as the risk of underlying anatomical or neuropathic disease is much greater than in NE and management is different (see below).

Behavioral Disorders

Behavioral/psychiatric disorders, such as oppositional defiant disorder, autism spectrum disorder (ASD), emotional symptoms, and in particular attention deficit/hyperactivity disorder (ADHD), are well documented as common comorbidities in NE children, especially in secondary NE and NMNE children [8, 14–16]. The clinical implications are important, as these children are more difficult to treat, show lower compliance, and have less favorable treatment outcomes [17]. Thus, approx. 20–30% of all NE children have at least one behavioral problem, which is twofold higher than in non-NE children [1, 11, 18, 19].

Sleep-Disordered Breathing

Sleep-disordered breathing (SDB) in children comprises a spectrum of sleep-related disorders from intermittent and habitual snoring to severe obstructive sleep apnea (OSA) with a prevalence of 1–5% [20]. Several studies have documented that NE is associated with SDB and OSA as well as asthma, allergic rhinitis, and atopic dermatitis [20–23]. The mechanism behind this is unclear but might involve increased nocturnal urine output [24] through vasoactive hormones and changes in sympathetic and parasympathetic nervous system activity [25, 26]. The clinical implications of SDB are still unclear as controversy exists about the effect of treatment of OSA on NE symptoms [27–29].

Consequences

Nocturnal enuresis is associated with severe negative psychological and social consequences leading to reduced quality of life, loss of self-esteem, social isolation, poor school performance in addition to punishment and domestic violence [30–32]. Interestingly, the reduced self-esteem scores have been shown to return to normal levels after successful treatment [33].

Pathogenesis

Nocturnal enuresis is a multifactorial condition that can be caused by one or more of several mechanisms and with a strong genetic component. A generally agreed pathogenic 3-factor model states that NE is caused by a mismatch between nocturnal urine volume and nocturnal bladder capacity together with an inability to awaken when this occurs (Fig. 1). This model is undoubtedly very simplified but has helped both physicians and families to better understand the nature of the condition.

Sleep/Circadian Rhythm Disturbances

The inability to wake up at the need to void has for ages been translated into the myth that NE children are deep sleepers. An increased arousal threshold to auditory stimuli has supported this hypothesis [34]. Using polysomnography to study sleep patterns in NE children several studies documented that NE usually occurs during the initial hours of sleep but can occur in all sleep stages, most commonly in stage N2, which is not considered deep sleep [35]. More recent cortical arousal analyses derived from sleep studies showed that NE children have a disturbed sleep with many transient arousals leading to sleep fragmentation and poor sleep quality [36–39]. One possible mechanism behind poor sleep quality is periodic limb movements during sleep (PLMS) that has been shown to occur more frequently in MNE with nocturnal polyuria [40, 41]. Interestingly, 6 months successful treatment of NE with the antidiuretic hormone analogue desmopressin was associated with reduced PLMS and improved neuropsychological function on several domains (e.g., working memory) as well as quality of life [42].

Circadian Rhythms

It is now well known that circadian rhythms coordinate our physiology at a fundamental level. The suprachiasmatic nucleus controls the

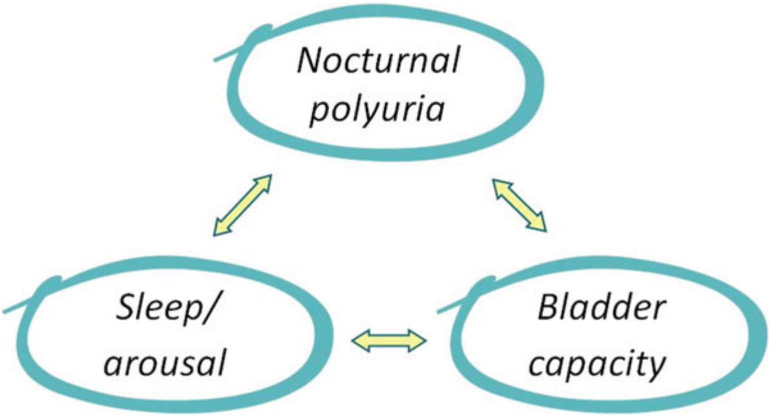

Fig. 1 The main three factors involved in the pathogenesis of nocturnal enuresis. In some children only one factor seems to contribute to the pathogenesis whereas others have two or more factors involved, for example, showing signs of both reduced nocturnal bladder reservoir function and nocturnal polyuria

circadian activity of other oscillators by neuronal and humoral pathways and many circadian clock genes participate in this complex regulation, for example, in bladder and renal tissue [43–45]. It has therefore been speculated that a disturbed circadian rhythm might be involved in NE pathogenesis.

Reduced Bladder Reservoir Function

Bladder reservoir function undergoes significant maturation during childhood involving volitionally control over micturition and an increasing bladder capacity by age until teenage years. There is accumulating evidence that bladder function undergoes circadian rhythmicity with an increased capacity during night, illustrated by the fact that the largest voids in most healthy children are the first morning voids [3]. A significant proportion of NE children (and especially of the NMNE subtype) has clear signs of reduced voided volumes during daytime compared with non-enuretic children, especially in NMNE children [46–51]. Furthermore, some enuretic children with normal voided volumes during daytime show reduced bladder capacity at night only [52]. The background behind this mechanism is not fully understood but may involve central nervous system regulation of bladder function [53].

Nocturnal Polyuria

As early as 1952 Poulton et al. demonstrated an abnormal circadian rhythm of urine output in NE with abnormally large diuresis at night-time [54]. Both in-patient circadian studies and home recordings of nocturnal urine volume later confirmed such defect and related this to a lack of circadian variation in plasma vasopressin with a lack of the normal nocturnal rise [55–61]. These studies showed that increased nocturnal urine output and low levels of plasma vasopressin are related to nights with enuresis and that patients showing this phenotype are characterized by a favorable response to treatment with the vasopressin analogue desmopressin [59]. The mechanisms behind nocturnal polyuria are multifactorial and include decreased urinary concentration [56], glomerular filtration [62], natriuresis [58, 63], and hypercalciuria [64–66]. A possible role of increased nocturnal arterial blood pressure [67–69] and sleep [63, 70] behind nocturnal polyuria in NE has been suggested. Figure 2 shows a possible pathogenic model behind nocturnal polyuria in NE. Future research may further clarify this complex area.

Genetic Factors

Nocturnal enuresis is a highly familial condition illustrated by the fact that 70% of NE children have a positive family history [71] and NE has an estimated twin based hereditability of 67% in boys and 70% in girls [72]. Furthermore, in large single-family studies positive linkage has been established in between NE and several gene loci (4p, 12q, 8q, 13q, and 22q), although no specific gene variations have been identified to date. Very recently, a comprehensive GWAS study including almost 35,000 individuals (3884 NE cases and 31,073 controls) identified six common variants in two different loci (chromosomes 6 and 13) [73]. These findings were replicated in a large Icelandic population and explained approx. 30% of the NE hereditability. Interestingly, the function of the potential risk genes identified (e.g., PRDM13) was related to sleep, urine production, and bladder function.

A common pathogenic hypothesis explaining the possible interrelationships between sleep and arousal, circadian rhythms, bladder function, and urine output regulation is presented in Fig. 2. As all of these mechanisms are under central nervous system control and as most of the involved centers are located in the pontine/hypothalamic region, it is possible that the NE phenotype is defined by dysregulation of one or more of these centers. Future research will test such hypothesis further.

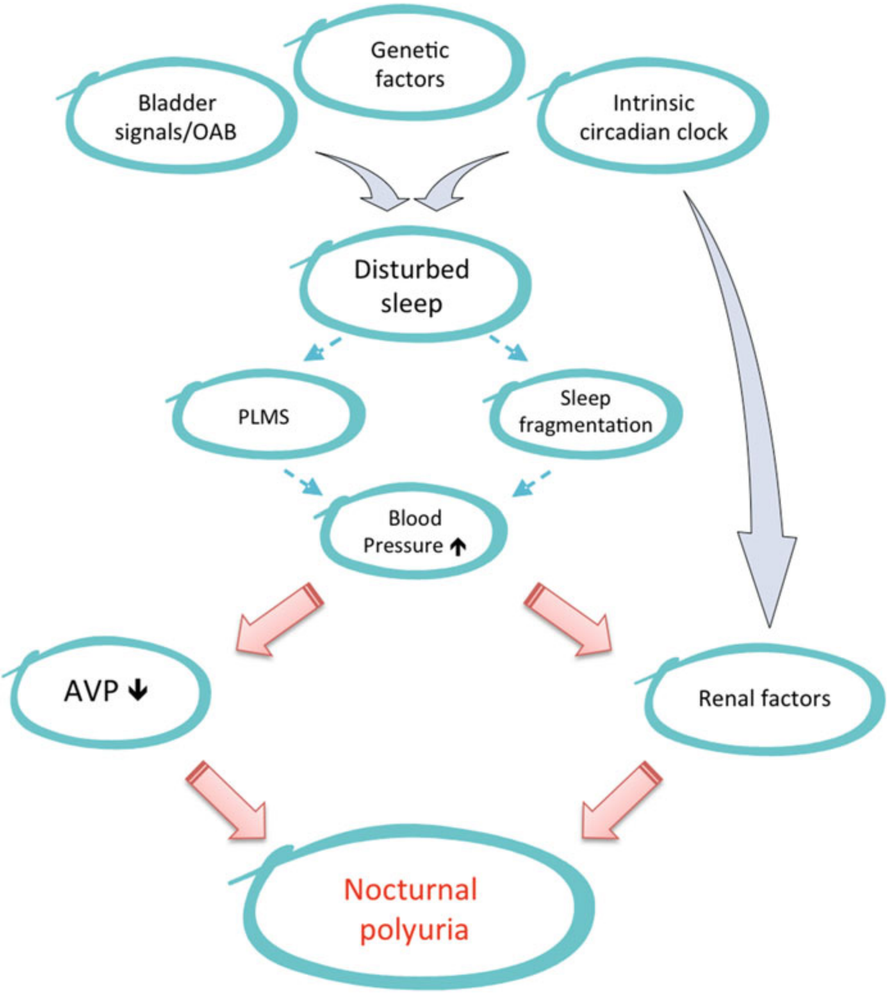
Genetic factors
Bladder signals/OAB
Intrinsic circadian clock
Disturbed sleep
PLMS
Sleep fragmentation
Blood Pressure ↑
AVP ↓
Renal factors
Nocturnal polyuria

Fig. 2 A proposed pathogenic model illustrating the complex background behind nocturnal polyuria in children with NE. Common risk gene variations (e.g., in PRDM13) may increase the risk of dysregulated sleep, circadian rhythm regulation, and bladder dysfunction during night, which can be visualized in sleep studies as PLMS and sleep fragmentation. This can lead to increased blood pressure levels during night resulting in pressure natriuresis and suppression of AVP secretion. Changed autonomic output from the suprachiasmatic nucleus to the kidneys (and other organs) might contribute to the increased urine output during night

Evaluation

History Taking

Several international guidelines and consensus reports exist on the evaluation and treatment of NE [1, 2, 74–78]. In the uncomplicated child with primary NE without the following warning signs:

- UTI
- Constipation
- Severe daytime voiding symptoms such as weak stream and straining (urethral valve)
- Recent onset of secondary enuresis (diabetes mellitus)

Evaluation should be undertaken when the child and family is motivated, usually before school start at 5–6 years of age. History taking is a very important part of NE evaluation aiming to characterize the patient subtype (e.g., MNE vs. NMNE), identify warning signs of underlying disease and other types of incontinence that need to be managed before NE, as well as other comorbidities. Finally, it is important to clarify the social and psychological impact of NE on the child and family and the motivation for therapy. The history should focus on severity and duration of the night wetting, the presence of daytime LUT symptoms (urgency, frequency (>7/day), incontinence, holding maneuvers, straining, interrupted or weak stream), drinking and voiding habits, UTI, urinary tract anomalies, constipation (Rome IV criteria) [79], fecal incontinence, and comorbid psychological disorders (e.g., ADHD). Also, the outcome of previous treatment attempts should be explored. Some guidelines provide checklists for history taking and physical examination [76, 77].

Physical Examination and Other Diagnostic Procedures

In patients with primary MNE without any warning signs (see above) the physical examination is often normal and not revealing [2, 80]. In all other cases or in treatment-resistant patients physical examination should be performed focusing on general health, genital examination, and for signs of occult spinal dysraphism (lumbosacral findings, leg and gluteal cleft asymmetries, abnormal neurology of lower extremities). If the history reveals positive signs of constipation a digital rectal examination (or ultrasound determination of rectal diameter [81]) should be considered, as rectal impaction is an important Rome IV criterion. A urine dipstick examination is only indicated if history or physical examination gives suspicion of underlying disease such as diabetes, UTI, or renal disease. Imaging such as ultrasound of kidneys and urinary tract, voiding-cysto-urethrography, MRI of lower back, and urodynamic investigations are generally not indicated in simple NE but should be reserved for specific patients only.

Diaries

Diaries are very useful tools in the evaluation of children with incontinence including NE, where they can help distinguish MNE from NMNE, reveal inappropriate drinking and voiding habits, and diagnose nocturnal polyuria. All of these might help the healthcare provider to select treatments targeting the underlying mechanism in each child. Ongoing prospective studies are investigating whether such tools indeed result in more favorable treatment outcomes. If such tools are omitted in the initial phase of management, they should be performed in complicated patients, for example, treatment resistance.

Frequency-Volume Charts

In order to estimate daytime bladder function and drinking behavior during daytime a 48-h frequency-volume chart should be performed [1, 82]. This is usually performed during a weekend and record forms can be downloaded from various websites or families can choose to use App versions where recordings can be made directly (e.g., “DryDawn App”). From this tool number of voids per day, maximum and average voided volumes, and total diuresis can easily be calculated. It should be noted, that if MVV is compared to age-related reference values (see section “Reduced Bladder Capacity”), the first morning void should be omitted in the calculation to avoid overestimation [3, 51].

Home Recording of Nocturnal Urine Volume

It is possible with relatively simple measures to estimate nocturnal urine volume in a home setting by weighing the dry diaper at bedtime and repeat this in the morning together with measuring volume of the first morning void. Adding the first voided volume to the difference in diaper weight is a rough but reliable estimate of nocturnal urine volume [82]. This recording should be performed for at least seven nights to give a reliable estimate. It is important to emphasize that in order to diagnose nocturnal polyuria (see section “Nocturnal Polyuria”) only recordings on enuresis nights should be included as nocturnal urine output is usually only high during “wet” nights. The urine output estimates on “dry” nights can be used to identify patients with signs of reduced bladder reservoir function during night-time as such patients would experience enuresis despite low nocturnal urine volume [52].

Other Tools

As the comorbidity with behavioral disorders such as ADHD is rather high and as such comorbidity affects treatment outcome for some treatments and as this is often underdiagnosed, it is recommended to screen for such comorbidity. Abbreviated questionnaire screening tools that can be applied in practice has been suggested and validated [83, 84].

Treatment

First-Line Treatment

Treatment of NE in uncomplicated children can be undertaken in primary care or through school nurses provided that the children are screened for warning signs. After the initial evaluation there are alternative strategies to follow if the family is motivated for treatment. It is important to stress that in NMNE patients daytime symptoms as well as constipation and other comorbidities must be dealt with first (see ▶ Chap. 58, "Bladder Disorders") with the aim of converting the child into an MNE subtype.

Initial treatment for all children with NE should include general advice with explanation of the condition, demystification, and removal of guilt and shame. Also, although regular basic urotherapy has failed to show effect on NE symptoms [85], many would give advice on good voiding habits and fluid intake.

A simple initial strategy consists of a presentation of the assets and drawbacks of the two recommended first-line treatments, that is, the enuresis alarm [86] or desmopressin [87] letting the family decide which option to choose first. If such treatment fails the other first-line option is selected and if both these attempts fail combination of the two first-line treatments can be considered. In most studies, approx. two-thirds of NE patients would respond to such a strategy.

A more extended and personalized strategy involves performing bladder diaries in order to characterize the possible mechanisms behind NE in the individual patient, for example, reduced MVV and/or nocturnal polyuria [2, 76]. In children with reduced MVV and without nocturnal polyuria the alarm system would be the first choice whereas desmopressin would by selected to children with nocturnal polyuria and normal MVV [80]. In children with both factors present a combination of alarm and desmopressin could be tried.

Treatment Resistance

Treatment resistance to either of the first-line treatments can be caused by a number of reasons (Table 2) that should be explored before changing to another modality.

In case of treatment resistance without obvious reason and if no diaries have been performed, it can be of value to do so at this stage. If diaries have been made without treatment, it can be useful to repeat these during ongoing treatment do explore whether the treatment has obtained its effect (e.g., reduced nocturnal urine volume).

Table 2 Some common causes of treatment resistance to the two first-line enuresis treatments, enuresis alarm, and desmopressin

Treatment resistance to enuresis alarm	Treatment resistance to desmopressin
The patient has NMNE or comorbidity (e.g., ADHD)	The patient has NMNE (e.g., overactive bladder)
The patient has both reduced MVV and nocturnal polyuria	The patient has reduced bladder capacity at night
The parents are not waking up the child to an alarm	Lack of adherence to treatment
The alarm is not used every night	Insufficient dosage (e.g., to 240 μg melt)
The alarm has false positive events (e.g., sweating)	Intake later than 1 h before bedtime
The alarm goes off more than once per night	Large fluid/osmotic intake during evening
Alarm treatment was stopped too soon (<8 weeks)	Sleep-disordered breathing (e.g., OSA)

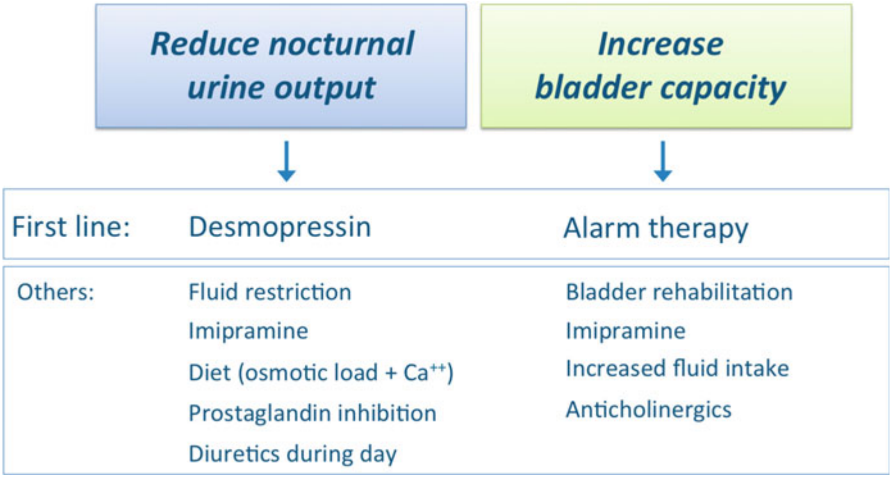

Fig. 3 The main two targets for nocturnal enuresis treatment reducing nocturnal urine output and increasing bladder capacity. The two recommended and evidence-based first-line treatments are desmopressin and the enuresis alarm. Other pharmaceutical treatment modalities include Imipramine, anticholinergics, prostaglandin inhibitors, and diuretics during daytime. Most of these treatments are associated with weak level of evidence. Also, increased fluid intake during daytime with an aim of increasing bladder capacity is advised worldwide despite minimal evidence

As shown in Fig. 3 most treatments for NE can be characterized as targeting either nocturnal urine volume or bladder reservoir function. As mentioned, the two most evidence-based treatments are desmopressin and enuresis alarm probably exerting their main effects on urine output and bladder capacity, respectively. There are, however, other treatment modalities influencing one or both of the two mechanisms and combination therapy using several modalities simultaneously have gained popularity despite the fact that evidence is absent or weak. A recent retrospective study in 59 children with refractory NE reported, however, that 15% showed partial (>50% reduction in wet nights) and 61% full response (>90% reduction) to such a strategy [88]. Imipramine has substantial evidence for efficacy but has potentially lethal cardiotoxicity with overdosing [89]. Anticholinergics have also shown efficacy usually in combination with desmopressin and have shown a more benign safety profile [90]. In most countries NE children refractory to first-line treatments are referred to secondary or tertiary care centers with particular interest and experience in evaluation and treatment of such patients.

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Pediatric Renal Tumors

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Abstract

Clinical aspects of pediatric renal tumors are presented addressing epidemiology, diagnostics, treatments, outcomes, and survivorship. The current chapter is inclusive of the most common pediatric renal tumor (Wilms tumor) as well as less common renal cancers and renal cancer predisposition syndromes.

Keywords

Wilms tumor · Renal Cell Carcinoma · Rhabdoid tumor · Clear cell sarcoma · Nephroblastomatosis

Introduction

Pediatric renal tumors encompass a wide spectrum of disease entities, both benign and malignant, affecting infants, children, adolescents, and young adults. Clinical presentation varies based on age, tumor differential, and extent of disease, but common presenting features include a painless palpable mass, hematuria, fever, anorexia and weight loss, constipation, abdominal/flank pain, and less commonly, respiratory compromise due to anatomical compression or metastatic disease. Care for such children warrants a multidisciplinary approach including but not limited to oncology, radiology, pathology, surgery, and occasionally radiation oncology. Nephrology-centered care may be relevant at any stage of a patient's journey, be it at diagnostics in a child with underlying renal disease undergoing routine scans, post-diagnosis on-therapy navigating underlying renal disease such as hypertension or renal insufficiency as well as consequences of nephrotoxic medications, or posttreatment for management of chronic renal disease, potentially evolving to end-stage renal disease. This chapter will focus on the epidemiology, pathophysiology and biology, radiology and staging approach, treatment, and survivorship issues of infant, children, and adolescents with renal tumors.

Epidemiology

Malignant kidney cancer accounts for nearly 7% of all pediatric cancer in the United States each year. By far the most common renal malignancy is Wilms tumor, accounting for 80% of all suspected renal masses noted on imaging and 90% of all pediatric renal cancers [1, 2]. Other relatively common malignancies include renal cell carcinoma, rhabdoid tumor of the kidney, clear cell sarcoma, and other sarcomas of the kidney. Metastatic tumors spreading to the kidney is rather uncommon in pediatric patients, especially when newly diagnosed with cancer, though not uncommon from Burkitt lymphoma. The chance of having a tumor other than Wilms tumor is higher in

Table 1 Pediatric renal tumors and age at diagnosis [14]

Renal tumor	Age (peak)
Congenital mesoblastic nephroma (CMN)	0–6 months
Ossifying renal tumor of infancy	1–3 months
Rhabdoid tumor of the kidney (RTK)	6–12 months
Wilms tumor (WT) – bilateral	15 months
Nephroblastomatosis	6–18 months
Cystic partially differentiated nephroblastoma	1–2 years
Clear cell sarcoma of the kidney (CCSK)	2 years
Wilms tumor (WT) – unilateral	3 years
Angiomyolipoma	10 years
Renal cell carcinoma (RCC; pediatric)	10–20 years
Renal medullary carcinoma	20 years
Cystic nephroma	Any
"Metanephric" adenoma/fibroma/ stromal tumor	Any

infants less than 6 months of age and children greater than 10 years of age [3, 4] (Table 1).

Benign tumors and tumors with less malignant potential involving the kidney occur at all ages, though are more common in infants and teenagers. Mesoblastic nephroma is by far the most common kidney tumor in newborns. Most patients are cured with surgery, including nearly all with stage 1 and 2 disease, though recurrences occur on occasion, and treated with medical and surgical therapy, leading many to consider such tumors as malignant [5–7]. Cystic nephroma (CN) and metanephric adenoma (MA) can present during childhood, with the potential for CN to be a presenting feature of the DICER1 cancer predisposition [8, 9] and may transform to or be associated with malignant components in some contexts [10, 11], warranting either surveillance or resection.

Investigations into associations of pediatric renal tumors with race have suggested an increased predilection for WT, for translocation renal cell carcinoma (tRCC), and for renal medullary carcinoma (RMC, typically associated with sickle cell trait) in patients of black or African descent [12–14]. Approximately 10% of all pediatric renal tumors overall are thought to occur in the setting of an identifiable cancer predisposition, with identifiable genetic predispositions linked to WT, RTK, CN, RCC, angiomyolipoma, and RMC [15, 16, 68]. Wilms tumor, at times bilateral (approximately 8% of all Wilms tumor) [17, 18] are often associated with overgrowth syndromes (most commonly Beckwith Wideman) [19, 20], WT1 mutation syndromes (Wilms tumor aniridia and growth delay (WAGD), Denys-Drash Syndrome (DDS) or Frazier Syndrome) [21–24], and dozens of other genetic conditions. Interestingly, only 10% of bilateral Wilms tumor cases are identified to be associated with known cancer predisposition conditions, raising question as to etiology of such cases, with undiagnosed genetic as well as developmental biology theories considered (refs above). Specific "familial Wilms tumor" genes have been identified, though account for <1% of all Wilms tumor cases overall [25, 26].

Radiology

The imaging evaluation of a suspected new abdominal mass depends on the patient's age and clinical presentation. In younger children, abdominal radiography and abdominal ultrasound are frequently utilized as the initial imaging tests to confirm the presence of a mass while avoiding the potential need for sedation, which may be required for computed tomography (CT) or magnetic resonance imaging (MRI). In older children and adolescents, ultrasound may still be utilized to evaluate specific clinical signs and symptoms, such as hematuria. However, these patients frequently have nonspecific clinical signs and symptoms and may undergo CT or MRI as their first imaging investigation (Fig. 1).

Abdominal Radiography

Abdominal radiographs in the setting of a suspected abdominal mass are of limited utility. Radiographs can demonstrate alternative diagnoses, such as severe constipation, which can sometimes present as a palpable abdominal mass. Displacement of bowel gas can be a clue that an abdominal mass is present and may indicate the likely organ of origin. For example, mass effect displacing bowel gas away from the left side of the

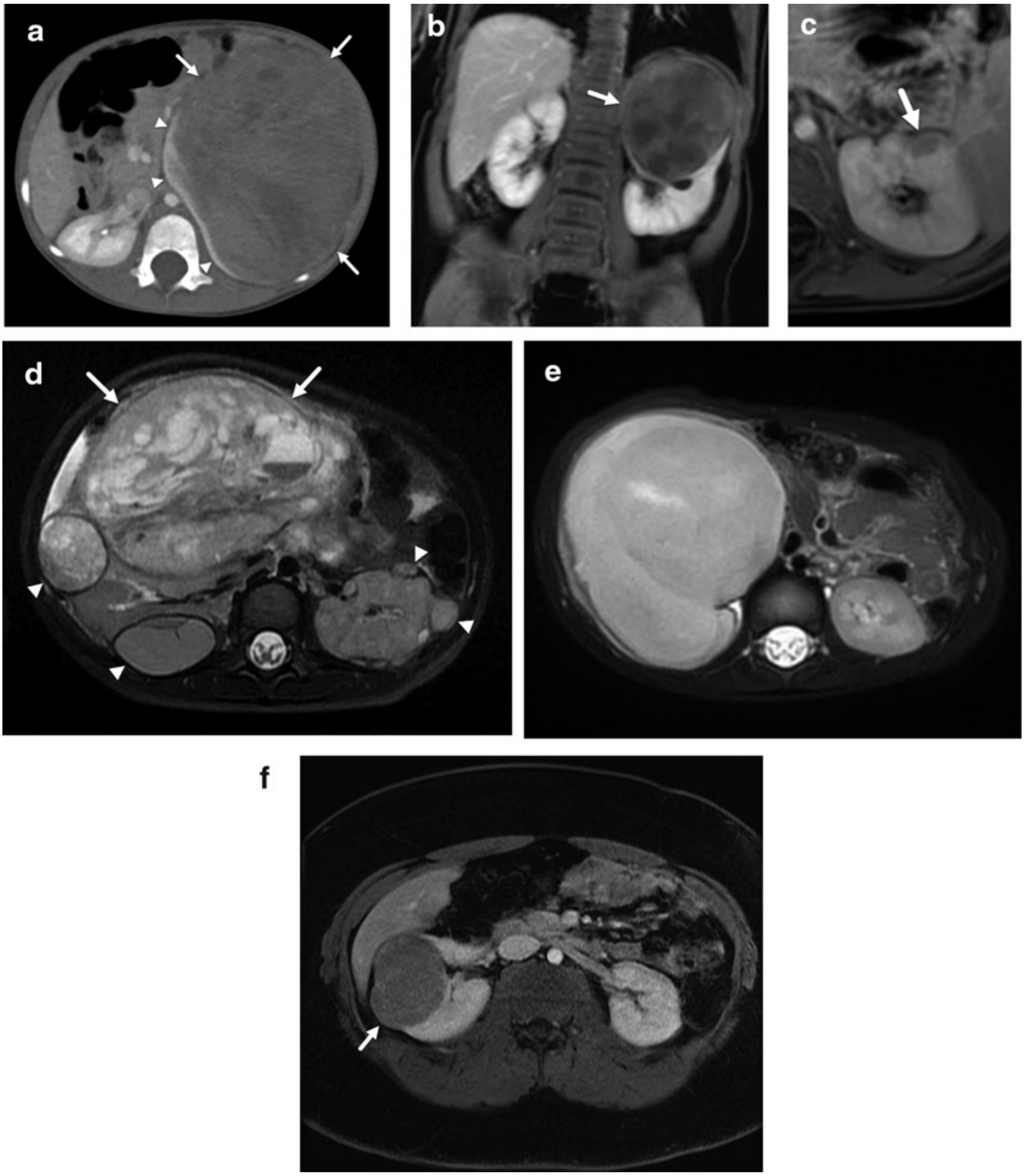

Fig. 1 Imaging features of various renal tumors. (**a**) Wilms tumor. Contrast enhanced CT images in a 3-year-old girl showing a large hypo-enhancing left renal mass (arrows) surgically proven to be a Wilms tumor. Note the "claw sign" with some normal enhancing renal tissue surrounding portions of the mass (arrowheads). (**b**) Wilms tumor. Coronal T1 weighted post contrast images in a 13-month-old boy show a large left upper pole renal mass (arrow) consistent with Wilms tumor. (**c**) Nephrogenic rest. Axial T1-weighted post contrast image in an 18-month-old boy shows a small, homogeneously hypo-enhancing lesion in the left kidney most consistent with a nephrogenic rest (arrow). (**d**) Bilateral Wilms tumor. Axial T2 weighted fat saturated image in a 3-year-old girl show a large heterogeneous right renal mass (arrows) and multiple smaller solid masses in both kidneys (arrowheads). (**e**) Clear cell sarcoma. Axial T2 weighted image in a 16-month-old girl show a large right renal mass which is relatively homogeneous in appearance. The patient underwent right nephrectomy and at pathology the mass was found to be a clear cell sarcoma. (**f**) Renal cell carcinoma. Axial T1 weighted post contrast image in a 14-year-old boy shows a hypo-enhancing, relatively homogeneous right renal mass confirmed to be renal cell carcinoma at resection

abdomen in a younger child most likely indicates a tumor of either left kidney or left adrenal gland origin, while displacement of bowel from the right side of the abdomen could be due to a tumor of the right kidney, right adrenal gland, or liver. Radiographs may demonstrate calcifications, which are more common in certain types of tumors (e.g., neuroblastoma), but are generally not specific. Finally, abdominal radiographs often include a portion of the lung bases. If present, the finding of pulmonary metastases may help to refine the differential diagnosis.

Ultrasonography

Ultrasound should be strongly considered as the initial imaging test in the work up a suspected renal mass or other abdominal mass, especially in younger children. Ultrasound is readily available, is relatively inexpensive, and does not require sedation, intravenous contrast, or ionizing radiation. Ultrasonography can confirm the presence of a mass, and can also exclude alternative diagnoses, such as congenital abnormalities (e.g., a congenital ureteropelvic junction obstruction in a neonate) or infection (such as xanthogranulomatous pyelonephritis). Importantly, ultrasound is also very useful to define the organ of origin of a mass, which may dictate the choice of CT versus MRI as the next imaging test. It is important to note, however, that ultrasound alone is not adequate for staging or for complete tumor characterization, and almost invariably CT or MRI is still required as a next step.

Computed Tomography

Computed tomography (CT) in the setting of a suspected renal mass allows adequate assessment of the primary mass, including size, multifocality, vascular invasion, and complications such as collecting system obstruction. In addition, CT can demonstrate retroperitoneal lymph node enlargement, extrarenal extension, and liver metastases. CT of the chest is the test of choice to evaluate for pulmonary metastases, and can also readily demonstrate intrathoracic lymph node enlargement, if present. The drawbacks of CT imaging include the use of ionizing radiation, the need for intravenous contrast, and the frequent need for sedation in younger children. Importantly, and in contradistinction to some adult CT protocols, a single-phase CT obtained in the portal venous phase is adequate to stage pediatric renal tumors [27]. Routine performance of pre-contrast and delayed imaging adds unnecessary radiation and are rarely, if ever, of diagnostic utility [28].

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) has been shown to have equivalent performance compared to CT in terms of the initial assessment of a suspected renal mass in a child [27, 29]. MRI avoids the use of ionizing radiation, but also more frequently requires sedation due to substantially longer imaging acquisition times. Both CT and MRI require intravenous contrast administration. Abdominal MRI may be preferred to CT in certain situations, such as suspected bilateral Wilms tumor. MRI may also be more sensitive for liver metastases and may be used as a problem-solving tool in the setting of equivocal findings of renal vein or inferior vena cava thrombus, as the MRI allows more complete assessment of the vasculature due to superior contrast resolution and the ability to perform multiphase post contrast imaging without the penalty of ionizing radiation. CT is superior to MRI for evaluation of pulmonary metastases.

Nuclear Medicine

Nuclear medicine plays a limited role in the diagnosis and work up of pediatric renal tumors. Assessment of split renal function may assist in surgical planning, especially in the setting of bilateral tumors. FDG-PET typically does not play a substantial role in the evaluation of pediatric renal tumors, except in the setting of late recurrent disease or other rare instances.

Imaging Features of Specific Tumor Types

There is substantial overlap in the imaging features of several of the most common pediatric renal tumors, and frequently patient age and clinical features play a significant role in ordering the differential diagnosis. However, there are some more typical features of certain tumor that may allow a more confident imaging diagnosis.

Wilms Tumor

Wilms tumor typically presents as a large, heterogeneous renal mass. The mass typically demonstrates a "claw sign" – a rim of normal renal tissue that "holds" on to the mass like a claw – which confirms the mass is of renal origin. On ultrasound, the Wilms tumor is usually relatively hypoechoic, but is frequently heterogeneous [30]. With Doppler, the mass frequently demonstrates decreased blood flow relative to the adjacent normal renal parenchyma. US with Doppler can also be used to assess the renal vein and inferior vena cava for tumor thrombus.

With contrast-enhanced CT and MRI, Wilms tumor is seen as a hypo-enhancing mass, usually centered in the renal parenchyma. At MRI, the mass is typically relatively hyperintense on T2 weighted imaging and frequently has areas that show restricted diffusion [30]. Frequently, the mass will have heterogeneous areas of enhancement in the periphery with central hypo-enhancement suggesting necrosis. Involvement of the collecting system is rare but can occur. Both CT and MRI are adequate for evaluation of vascular tumor thrombus [31]. Calcifications, better seen on CT than on MRI, do occur in Wilms tumor but are relatively rare [30]. CT and MRI do not reliably visualize the tumor capsule or extracapsular spread [32]. The sensitivity and specificity of CT and MRI for tumor rupture are also limited. A moderate amount of free fluid, extending above the pelvis, was shown to be the only imaging feature associated with rupture [31]. Tumor rupture cannot be excluded by imaging.

In the setting of bilateral Wilms tumor, MRI with gadolinium containing intravenous contrast is often the preferred imaging modality [27], as MRI may be more sensitive for detection of multifocal lesions. In addition, as these patients typically undergo neoadjuvant chemotherapy prior to any surgical intervention, MRI allows for repeated assessments of treatment response without the exposure to ionizing radiation.

Nephrogenic Rests

Nephrogenic rests are small foci of residual metanephric blastema that persist beyond 36 weeks of gestation [30]. Perilobar nephrogenic rests are more common but have a lower risk of malignant degeneration than intralobar nephrogenic rests [30]. Nephrogenic rests can be difficult to see on US, but may manifest as small oval shaped or round hypo-echoic lesions. On CT and MRI, nephrogenic rests typically appear as small hypo-enhancing lesions, often in the periphery of the kidneys [30]. Unfortunately, imaging cannot reliably distinguish nephrogenic rests from small Wilms tumors. Larger size (over 1.75 cm in diameter) has been shown to be associated with a higher likelihood of Wilms tumor [33]. Additionally, nephrogenic rests tend to be more homogeneous and often have an oval or lenticular shape. Diffusion weighted MRI has been suggested as a possible tool to distinguish between nephrogenic rests and small Wilms tumors, but to date no studies have proven the utility of diffusion weighted imaging in this context [27].

Mesoblastic Nephroma

Imaging features of mesoblastic nephroma can be identical to those of Wilms tumor, typically presenting as a large heterogeneous renal mass [30]. The patient's age at presentation is the most important feature in ordering the differential diagnosis – mesoblastic nephroma is more common in patients under 6 months of age, whereas Wilms is much more likely if the patient is over 1 year of age. The presence of distant metastases and tumor thrombus are also much more likely in Wilms tumor than mesoblastic nephroma.

Clear Cell Sarcoma

These tumors typically manifest as a large, heterogeneous mass that is hypo-enhancing relative to normal renal parenchyma. Clear cell sarcoma may have a more cystic appearance than a typical Wilms tumor on CT. Calcifications are rare but are more common in clear cell sarcoma than in Wilms tumor [30]. In addition, bone metastases are more common in clear cell sarcoma than in Wilms tumor. MRI and nuclear medicine bone scan or PET scan are more sensitive for bone metastases than CT.

Rhabdoid Tumor

At CT and MRI, rhabdoid tumor most commonly appears as a large, heterogeneous mass, more frequently located centrally within the kidney. An imaging feature described more commonly with rhabdoid tumor is the presence of subcapsular fluid, although this finding is neither sensitive nor specific [34, 35]. Calcifications are more common than in Wilms tumor [35]. An association with synchronous and metachronous primary central nervous system tumors has been described.

Multilocular Cystic Renal Tumor

The term multilocular cystic renal tumor encompasses both cystic nephromas, a benign tumor, and cystic partially differentiated nephroblastoma, which contains immature blastemal elements. At ultrasound, these tumors are typically large, with multiple cystic spaces separated by thin septae. Calcifications are rare but do occur [8, 30]. CT and MRI both demonstrate similar findings, with a large multilocular cystic mass divided by thin, uniformly enhancing septa [8]. Solid components are rare, and more likely in the cystic partially differentiated nephroblastoma variant. Multilocular cystic renal tumors frequently have well-defined margins and may have a visible capsule [8, 30]. The tumors have a propensity to “herniate” toward the renal pelvis and collecting system [8].

Renal Cell Carcinoma

Renal cell carcinoma (RCC) is more common in older children and adolescents. In distinction to Wilms tumor, these lesions are frequently smaller in size at presentation, but can still occasionally be quite large. On US, RCC most commonly appears as a round, mildly heterogeneous and hypo-echoic mass. At CT and MRI, the lesions are typically hypo-enhancing relative to normal renal parenchyma and may be exophytic [35]. Heterogeneity may be due to hemorrhage or necrosis. Peripheral or rim-like calcifications may be present [35]. On MRI, RCC is usually iso-intense to mildly hyperintense on T2 weighted imaging, but is frequently heterogeneous, and may demonstrate restricted diffusion [35]. Tumor thrombus can occur and is readily identified on both CT and MRI. Metastatic disease is most commonly seen in retroperitoneal lymph nodes, liver, lungs, bone, and brain.

Renal Medullary Carcinoma

Renal medullary carcinoma is a highly malignant tumor that occurs in adolescents and young adults with sickle cell trait or hemoglobin SC disease. At imaging, the tumor typically appears as an aggressive, infiltrative mass located centrally within kidney. These tumors are typically more irregular shaped and frequently infiltrate the renal hilum, as compared to the more typically round masses seen with Wilms tumor and RCC [36, 37]. Renal medullary carcinoma may infiltrate the collecting system and ureter, and retroperitoneal lymph nodes are frequently involved [35]. Metastatic disease to the lungs is common and may have a lymphangitic pattern as opposed to discrete lung nodules [37].

Screening and High-Risk Populations

In patients with genetic syndromes that predispose to renal tumors, imaging screening is usually accomplished using abdominal or renal ultrasound. While less sensitive and specific than CT or MRI, ultrasound eliminates the need to intravenous access, sedation, and exposure to ionizing radiation (with CT). For example, in hemihypertrophy syndromes such as Beckwith-Weideman Syndrome, patients are typically screened with ultrasound every 3 months for the first 7–8 years of life [19]. Due to the relatively high false positive rate of screening US, an abnormal screening US should be followed by a contrast enhanced CT or MRI. In older children with cancer predisposition syndromes such as Von-Hippel-Lindau, screening with whole body MRI is becoming increasingly more common (Table 2).

In terms of surveillance imaging in patients with a history of a renal malignancy, for the majority of WT patients, chest radiography and ultrasound of the abdomen are replacing traditional use of CT and MRI, based on equivalent survival at relapse independent of modality used [38]. For other renal tumors, CT chest and CT or MRI abdomen remain the imaging tests of choice.

Staging Pediatric Renal Tumors

Pediatric renal tumors, aside from renal cell carcinoma (RCC), share a common pathologic staging system in the United States which differs from that used for renal tumors in adults and is slightly different from the staging system used by SIOP in Europe [39]. This system focuses on the natural history and characteristics of the most common tumor, Wilms' tumor (WT). Most notably, bilateral tumors are assigned a unique stage V with local sub-staging for each kidney. In addition, the friability of WT is such that a biopsy of the tumor prior to nephrectomy automatically merits a Stage III, which is otherwise used for tumor spillage during resection, positive resection margins, and other situations in which tumor is likely to remain within the patient after resection. Stage I, in which tumor is confined to the kidney, Stage II, in which tumor has breached the kidney capsule or infiltrated the renal sinus soft tissues, and Stage IV, in which tumor extends into adjacent organs via hematogenous spread or distant metastasis is observed, are similar to the pathologic staging of other tumors. Details of the Children's Oncology Group (North America) and International Society of Pediatric Oncology (Europe) staging systems for WT are detailed in Table 3.

Table 2 Pediatric renal tumor cancer predisposition syndrome and recommended surveillance

Tumor	Implicated gene(s)	Syndrome	Kidney tumor surveillance	Reference
Wilms tumor				
Overgrowth	11p15, SGB, other	Beckwith-Wideman	Ultrasound every 3 months through age 7 years	Kalish et al. [19]
WT1 and other	WT1, other	WAGR, DDS, Frazier, other	Ultrasound every 3 months through age 5 years	Hol et al. [22] (above)
Rhabdoid tumor	SMARCB1/ SMARCA4	Rhabdoid tumor predisposition	Suggest ultrasound every 3 months through age 5 years + other anatomical targeted imaging	Foulkes et al. [40]
CN (WT, renal sarcoma)	DICER1	DICER1 cancer predisposition	Ultrasound every 6 months until 8 years of age then every 12 months until 12 years of age	Schultz et al. [41]
RCC clear cell RCC; SDHB-associated RCC	VHL; SDHB	Von Hippel-Lindau; hereditary paraganglioma	Annual ultrasound or MRI starting at age 16 (VHL) + other targeted imaging; not established (SDHB)	Chahoud et al. [42]; VHL Alliance
Angiomyolipoma, PEComa/RCC	TSC1/TSC2	Tuberous sclerosis	MRI at diagnosis and then every 1–3 years	Krueger et al. [43]
RMC	Hemoglobinopathy	Sickle cell trait	Not established	

Table 3 Staging pediatric renal tumors

Stage	Children's Oncology Group (COG)	International Society of Pediatric Oncology (SIOP)
I	(a) Tumor is limited to the kidney and completely excised (b) The tumor was not ruptured before or during removal (c) The vessels of the renal sinus are not involved beyond 2 mm (d) There is no residual tumor apparent beyond the margins of excision	(a) The tumor is limited to kidney or surrounded with fibrous pseudocapsule if outside of the normal contours of the kidney. The renal capsule or pseudocapsule may be infiltrated with the tumor but does not reach the outer surface and is completely resected (resection margins "clear") (b) The tumor may be protruding into the pelvic system and "dipping" into the ureter (but it is not infiltrating their walls) (c) The vessels of the renal sinus are not involved (d) Intrarenal vessel involvement may be present
II	(a) Tumor extends beyond the kidney but is completely excised (b) No residual tumor is apparent at or beyond the margins of excision (c) Tumor thrombus in vessels outside the kidney is stage II if the thrombus is removed en bloc with the tumor *Although tumor biopsy or local spillage confined to the flank were considered stage II by NWTSG in the past, such events have been considered stage III in COG studies*	(a) The tumor extends beyond kidney or penetrates through the renal capsule and/or fibrous pseudocapsule into perirenal fat but is completely resected (resection margins "clear") (b) The tumor infiltrates the renal sinus and/or invades blood and lymphatic vessels outside the renal parenchyma but is completely resected (c) The tumor infiltrates adjacent organs or vena cava but is completely resected
III	Residual tumor confined to the abdomen (a) Lymph nodes in the renal hilum, the Periaortic chains, or beyond are found to contain tumor (b) Diffuse peritoneal contamination by the tumor (c) Implants are found on the peritoneal surfaces (d) Tumor extends beyond the surgical margins either microscopically or grossly (e) Tumor is not completely respectable because of local infiltration into vital structures	(a) Incomplete excision of the tumor which extends beyond resection margins (gross or microscopic tumor remains postoperatively) (b) Any abdominal lymph nodes are involved (c) Tumor rupture before or intraoperatively (irrespective of other criteria for staging) (d) The tumor has penetrated though the peritoneal surface (e) Tumor implants are found on the peritoneal surface (f) Tumor thrombi present at resection margins of vessels or ureter, transected or removed piecemeal by surgeon (g) The tumor has been surgically biopsied (wedge biopsy) prior to preoperative chemotherapy or surgery
IV	Presence of hematogenous metastases or metastases to distant lymph nodes	Hematogenous metastases (lung, liver, bone, brain, etc.) or lymph node metastases outside the abdominal-pelvic region
V	Bilateral renal involvement at the time of initial diagnosis	Bilateral renal tumors at diagnosis. Each side should be sub-staged according to the above criteria

Pathology

Wilms' Tumor (Nephroblastoma)

Wilms' tumors (WT) are most commonly unicentric, grossly well-demarcated, and often encapsulated on gross examination, commonly arising from either pole of the kidney rather than the midline. They are generally large and roughly spherical in shape, causing distortion to the renal parenchyma. The cut surface is white-tan to gray in color, soft, friable, and characteristically bulges above the surface once cut. There are often hemorrhagic and necrotic foci, and cystic change can range from a minor component to a dominant feature. Because the tumor is so friable, displacement of tumor cells into spaces or onto the surface of the tumor via "knife metastasis" is nearly inevitable unless proper handling techniques are used [44].

Classic WT are triphasic, composed of an admixture of three histologic elements: primitive, round cells with high nucleus:cytoplasm ratio (the blastema), epithelial elements (predominantly recapitulations of developing kidney such as immature tubules and glomeruloid structures, but includes heterologous epithelium such as squamous), and stroma, which may be relatively undifferentiated spindled cells in a loose myxoid background, or heterologous stromal elements, particularly striated muscle. The tumor may be described as "predominant" in one of these elements if it constitutes >67% of the tumor, or as simply mixed or triphasic. If the classic elements are present, there are few histologic mimics or differential diagnoses for this tumor, though blastema predominant tumors must sometimes be differentiated from lymphomas, neuroblastomas, and other small blue cell tumors that may affect the kidney on biopsies, and other differentials must be considered if pseudo-rhabdoid morphology, rhabdomyomatous differentiation (Fig. 2), or cellular stromal elements dominate.

In approximately 5–9% of cases, anaplasia is found within these tumors. Anaplasia may be defined in WT as nuclei enlarged to three or greater times the size of adjacent nuclei, hyperchromasia, and large atypical mitoses which are suggestive of an increase in chromosomal content. These findings may be seen in any of the three histologic components, though stromal anaplasia alone is rare. Increase in typical mitoses, karyorrhexis, and nuclear enlargement insufficient for anaplasia may be termed "nuclear unrest," which by itself does not carry the same poor prognostic implications as anaplasia. Focal anaplasia is defined as one or more foci with anaplasia which are well defined by surrounding non-anaplastic tumor and no background nuclear unrest. Diffuse anaplasia is characterized by random, poorly defined foci of anaplasia spread throughout the entirety of or a large, undefined area of a WT. Any anaplasia in the presence of background nuclear unrest, or anaplasia in a needle biopsy, metastasis, or extrarenal site is defined as diffuse anaplasia automatically.

Nephrogenic Rests/Nephroblastomatosis

Nephrogenic rests are believed to be remnants of immature/primitive renal tissue which persists beyond approximately 36 weeks gestation when mature renal tissue alone is expected. Perilobar nephrogenic rests (PLNR) are located at the periphery of the lobe (the unit of renal development) and are often well demarcated with predominantly blastemal or epithelial morphology. Frequently these are multiple (known as nephroblastomatosis), often numerous around the subcapsular zone. Intralobar rests (ILNR) are often poorly demarcated, infiltrative lesions in the deep cortex or medulla. These are more likely to be solitary and contain tubular and glomeruloid structures in addition to blastema and stromal elements. Nephrogenic rests are considered precursor lesions to WT, and are noted in 30–40% of WT cases, with up to 98% of cases of multifocal WT arising from NR. Further characterization of both types of NR into dormant/incipient, regressing/sclerosing, and hyperplastic NR may be done based on their histologic appearance, with hyperplastic NR particularly difficult to distinguish from true WT. A true tumor capsule, if seen, is more characteristic of a WT.

Genetics of Wilms Tumor/Nephrogenic Rests/Nephroblastomatosis and Syndromic Associations

Tumor-specific cytogenetic changes, particularly loss of heterozygosity (LOH) for chromosome 1p and 16q and gain of 1q, have been shown to be important prognostic factors which impact overall risk stratification and chemotherapy protocol [45–47]. Investigation of tumors at diagnosis by SNP array or alternative technique is therefore an important element of proper workup.

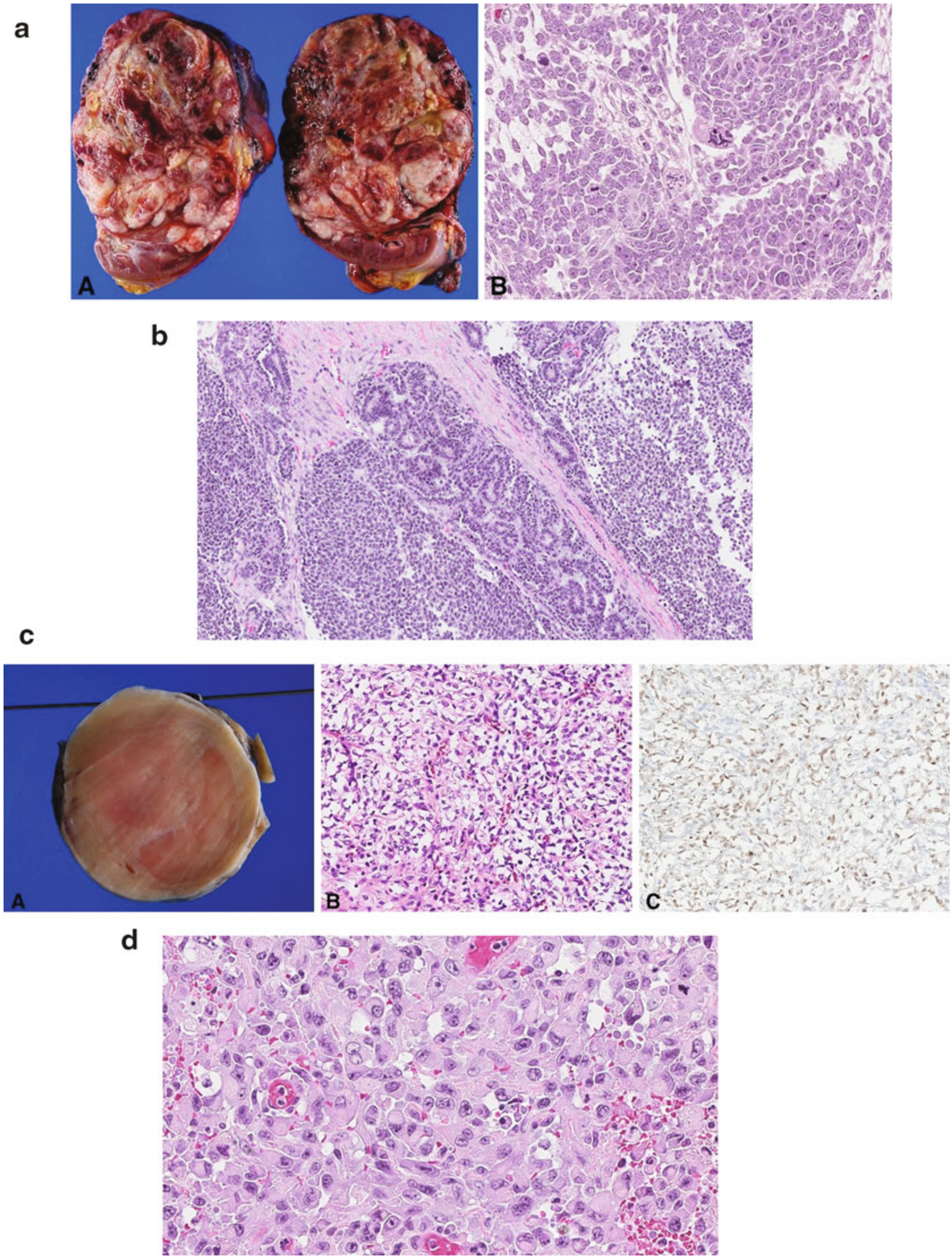

Fig. 2 (continued)

Constitutional perturbations in WT1 and 2p24.3 duplication have been linked to arrest in development of embryonic renal tissue resulting in the development of nephrogenic rests. Subsequent events likely facilitate oncogenesis to a more malignant phenotype. The pathogenesis of WT is complex, however [48]. Greater than 20 WT constitutional/familial predisposition genes, reflecting diverse biological processes, have now been identified, of which the most common are *TRIM28, REST, WT1, CTR9, H19* hypermethylation, *CDC73*, Biallelic *BRCA2*, and Biallelic *NYNRIN*. Additional genes include *ASXL1, DICER1, KDM38, TP53, BLM, BUB18, DIS3L2, PALB2, TRIM37, TRIP13, GPC3* (X-linked), and *Pik3CA* (mosaic) [25]. Subsequent sporadic genetic events in WT resulting in putative driver mutations include but are not limited to mutations impacting nephron differentiation (*CTNNB1, AMER1, MLLT1, SIX1,* and *SIX2*) and mutations affecting microRNA (miRNA) production (*DROSHA, DGCR8*, and *XPO5*) [49, 50]. TP53 mutation, which is more commonly sporadic, is particularly important, as it has been associated strongly with anaplastic histology and may be localized and assessed with immunohistochemistry [51, 52]. For a more complete review of the complex genetic and epigenetic landscape of WT, its pathogenesis, and relationships to renal development, the reader is referred to a current review by Li et al. [53].

Congenital Mesoblastic Nephroma

Congenital mesoblastic nephromas (CMN) are generally large, gray-tan solitary tumors with a soft, whorled appearance to the cut surface. They are poorly defined and appear infiltrative even on gross examination, particularly into the soft tissues of the renal hilum. Completeness of surgical resection is an important prognostic factor so careful examination, particularly of the hilum, is important. By histology, classic CMN are composed of fascicles of relatively bland spindle cells, occasionally showing collagen infiltration. Cysts may be seen, and the mitotic activity is generally low. The tumor is particularly distinctive at the periphery, where tongues of cells extend and infiltrate the surrounding tissue, frequently entrapping native renal structures. Cells of similar morphology but higher mitotic rate with higher cellularity and crowding, resembling infantile fibrosarcoma, constitute the cellular variant of CMN. The cells appear fibroblastic or myofibroblastic on H&E and show positivity for muscle actins and desmin. This tumor may resemble a stroma predominant Wilms tumor, which is less common in the first 2 years of life when CMN is common. It may also be differentiated from clear cell sarcoma of the kidney through subtle features such as entrapping islands of parenchyma at the periphery rather than individual structures. Monophasic synovial sarcoma has a classic immunohistochemical pattern and cytogenetic driver that allows it to be distinguished from CMN.

The cytogenetic driver of this tumor, particularly the cellular variant, is the t(12;15)(p13; q25) translocation producing ETV6-NTRK3 fusion identical to that seen in infantile fibrosacoma. The cellular variant is also frequently aneuploid, with additional copies of chromosomes 8, 11, and 17. The classic variant, on the other hand, shows no characteristic genetic abnormality [54–56].

◀

Fig. 2 (**a**) Favorable histology Wilms tumor (H&E, 170× magnification) – all three components of the classical triphasic WT are seen, including blastemal, epithelial, and stromal elements. Wilms tumor grossly is encapsulated and friable, with foci of hemorrhage and necrosis often seen. (**b**) Anaplasia in a WT (H&E, 400× magnification) is characterized by massive nuclear enlargement and large, bizarre atypical mitotic figures, seen here. (**c**) Clear cell sarcoma of the kidney is grossly well circumscribed without a capsule, and fibrous and firm in texture. CCSK (H&E, 300× magnification) shows classic histology with clearing of both cytoplasm and nuclei, in nests defined by a fine network of capillaries. Positive nuclear staining for BCOR by immunohistochemistry is a common finding. (**d**) Rhabdoid tumor of the kidney (H&E, 400× magnification) shows classic rhabdoid cytologic features, including eccentrically placed nuclei, prominent nucleoli, and eosinophilic cytoplasmic inclusions

Clear Cell Sarcoma of the Kidney

Clear cell sarcomas of the kidney (CCSK) often present as large (2–24 cm) masses that may, if small enough, appear centered in the medulla. They are circumscribed but unencapsulated and may show necrosis, hemorrhage, and cysts. Microscopically, this tumor shows a classic pattern of plump round-oval cells with scant or moderate amounts of pale or clear cytoplasm in nests and cords, separated by myxoid stroma with arborizing vessels. The tumor may also show areas of spindle cell morphology, epithelioid morphology, palisading, sclerosing, pericytomatous, and monstrocellular/anaplastic areas. The periphery of the tumor often appears “scalloped,” with a sense of circumscription at first glance but entrapping individual nephrons, often with cystic dilatation, when the periphery is examined more closely. The tumor shows variable enough morphology to mimic in at least some cases almost every other pediatric renal tumor, though the classic morphology may be found in most cases if sufficiently sampled and searched. It characteristically stains for vimentin, and cyclin D1 is a sensitive, if not specific, marker.

Investigations into the genetic alterations in CCSK show a BCOR internal tandem duplication in many cases and a t(10;17) translocation producing a YHWAE-NUTM2 fusion in other cases, which tend to be mutually exclusive [57–60]. The few remaining cases have rare BCOR-CCNB3 fusion or other rarer abnormalities, and specific immunohistochemistry targeting BCOR is commonly used as a correlate with abnormalities for rapid diagnosis [61].

Malignant Rhabdoid Tumor of the Kidney

Malignant rhabdoid tumors of the kidney (MRT) are roughly spherical with a soft, tan cut surface. Often these are grossly extensive beyond the kidney at diagnosis and have extrarenal metastases at presentation. The classic rhabdoid cytology, described as large epithelioid cells with abundant eosinophilic cytoplasm, a dense hyaline cytoplasmic inclusion a large pale/vesicular nucleus, and a prominent nucleolus, is a significant clue to the diagnosis on histology, though not pathognomonic for it. The tumor is unencapsulated with ill-defined borders and vascular invasion is common. The most common histologic mimic is renal medullary carcinoma, but this tumor may be recognized by the loss of INI-1 by immunohistochemistry.

The loss of INI-1 by immunohistochemistry is a correlate of the bilallelic inactivation of the SMARCB1/BAF-47 tumor suppressor gene on chromosome 22q11–12 that is the driver of these tumors. Since the same inactivation was described in atypical teratoid-rhabdoid tumors of the brain (ATRT), familial inheritance of a germline mutation has been described as the rhabdoid tumor predisposition syndrome. These patients may develop aggressive rhabdoid tumors, including those in the kidney, ATRT in the brain, and other SMARCB1 inactivated tumors such as epithelioid sarcoma of the soft tissue. Few cases of this tumor show inactivation of the related SMARCA4 gene, for which an immunohistochemical stain to detect the loss of the gene product BRG1 has been developed [62, 63].

Treatment

Surgery

Regardless of pathology, surgery is a key component of the management for children with renal tumors. Across pathologies, this often centers on early and complete surgical excision of the primary tumor, where possible. The timing of surgery – whether before or after initiation of chemotherapy – may vary depending on whether treatment occurs according to COG or SIOP protocols. The goals of surgery, across pathologies and COG/SIOP, are largely the same: safe removal of the tumor (with complete excision as possible), avoidance of intraoperative spill, and thorough lymph node sampling [64]. Among very-low risk Wilms tumors (children <2 years of age with stage I favorable histology Wilms tumors less than 550 g), surgery is the sole necessary initial therapy. These patients can be successfully observed without initial chemotherapy with

85% event-free survival and >98% overall survival [65]. There is potential to extend the “upfront nephrectomy followed by observation without chemotherapy” to additional WT subgroups [66].

As to lymph nodes, on study National Wilms’ Tumor Study-5 (NWTS-5), a surgical quality assurance program identified that lymph node sampling was not performed in 9% of WT cases, and that for 41% of stage III patients, nodal positivity was the sole factor that up-staged the patient to local stage III [67, 68]. The importance of lymph node sampling extends to other pathologies besides Wilms tumor as well [69, 70], including translocation-associated renal cell carcinoma (tRCC) which has high rates of nodal positivity [13, 71, 72]. Formal lymph node dissection, as opposed to sampling alone, in the management of pediatric RCC remains controversial, with some advocating for complete lymph node dissection while others believe it may not be indicated in the absence of suspicious imaging [73–75].

Particularly important among surgical considerations is when nephron-sparing surgery (NSS) should be utilized for patients with renal tumors, especially favorable-histology Wilms tumor. NSS is a key strategy for preservation of renal mass and function in these patients. Renal failure is rare amongst unilateral non-syndromic patients, with a rate of about 0.6%, but much more common as a long-term complication in patients with bilateral Wilms tumor (BWT) with rates as high as 11.5% [76]. BWT occurs in about 5–7% of patients with Wilms tumor [77]. AREN0534, a study protocol through COG, clarified the approach to these patients with preoperative chemotherapy and surgical intervention by no later than 12 weeks [78]. The use of bilateral NSS has increased in recent years, from rates of 13.8% of patients undergoing bilateral NSS on NWTS-2 and NTWS-3 to 19.4% with NWTS-4, and 35% with AREN0534. This strategy has largely been reserved for patients with BWT, Wilms tumor in a solitary kidney, or unilateral Wilms tumor (uWT) in patients with a predisposition syndrome. With an increase in use of the technique, there has also been an increase in awareness of complications. The most frequent complications after NSS include persistent urine leak, urinary tract infections/pyelonephritis, intestinal obstruction, and intussusception [79, 80]. Increasingly, given the excellent outcomes for favorable histology Wilms Tumor, efforts have shifted to minimizing the treatment-associated morbidity. It is in this context that consideration of NSS for unilateral Wilms Tumor has arisen. Numerous studies, including from SIOP, have demonstrated good results with NSS for uWT [81, 82]. However, these studies have demonstrated a relatively high rate of positive margins (58%) in this population, upstaging patients to local stage III with an impact on intensity of chemotherapy and radiotherapy, which indicates the importance of case selection and technical expertise [83]. Others have shown lower rates of positive margins (23.5%) with no association with local recurrence. Predictive algorithms suggest that only approximately 5% of unilateral FHWT present with features suggesting feasibility of a nephron sparing approach [84].

One key driver of NSS is the preservation of renal mass, and therefore renal function. One series of 17 patients, including 8 with bilateral NSS and 9 with unilateral nephrectomy and contralateral NSS demonstrated ESRD in 3 of 17 patients – including two who were rendered anephric due to further resections for recurrences [76]. All three patients had received radiation secondary to tumor stage or histology. Efforts to understand the impact of renal volume and function have found that patients undergoing NSS with ≥40% preservation of volume had no deterioration in their renal function during adolescence with preserved global renal function within normal values [85]. When comparing patients who underwent nephrectomy with those undergoing NSS (for unilateral Wilms tumor), those undergoing nephrectomy had an eGFR loss of 19.1 compared with an eGFR gain of 28.6 for those undergoing NSS [81]. These findings suggest that NSS results in improved renal function preservation and should be carefully considered in select patients with renal tumors, where surgical considerations and margin status will not compromise oncologic outcomes.

Medical Therapy

Chemotherapy standards exist for WT and CCSK, in both the COG and SIOP. Therapeutic intensity is based on risk stratification variables including tumor stage, histology (favorable vs anaplastic), biology (LOH 1p/16q), and response to initial therapy both radiologically and histologically [47, 86–94]. Using such approaches, many poor prognostic variables can be overcome including LOH 1p/16q, advanced stage in favorable histology Wilms tumor, and anaplasia in low stage patients [95]. Common chemotherapy agents used to treat WT and CCSK, as well as RTK, are listed in Table 4.

Targeted Therapy

With advances in biological understandings and emphasis on personalized targeted therapy, several renal-tumor specific therapies have emerged. First, targeting NTRK with TRK inhibitors is evolving as a potential replacement for chemotherapy for treatment of relapsed/metastatic mesoblastic nephroma [96]. Secondly, targeting mTOR with mTOR inhibitors has proven beneficial in patients with angiomyolipoma, particularly TS-associated [97]. Efforts to identify better biological risk stratifiers (such as gain of 1q [46]) targeted therapies for biologically unfavorable and relapsed WT, RTK, and tRCC are underway [98–100].

Radiation

Due to the relative radiosensitivity of Wilms tumor, radiation has been used for the treatment of both the primary site and metastatic sites. Primary site radiotherapy is guided by local stage as well as histologic considerations. NWTS 1 and 2 demonstrated lack of benefit for radiotherapy in early local stage tumors. NWTS-3 established 1000 cGy as equivalent to 2000 cGy in stage III FH patients when doxorubicin is included. Thus, for FH stage III WT, flank radiotherapy to 10.8 Gy in 6 fractions is standard of care. If there is evidence of peritoneal dissemination via preoperative rupture or intraoperative spillage beyond the tumor bed, then this transitions to 10.5 Gy in 7 fractions directed at the whole abdomen [101]. Results from AREN0321 demonstrate excellent local control rates with the use of

Table 4 Chemotherapy used to treat pediatric renal tumors

Drug	Mechanism	Tumors	Select common side effects (beyond alopecia/nausea/vomiting)
Vincristine	Anti-mitotic; inhibits microtubule assembly	Wilms, CCSK, rhabdoid	Peripheral neuropathy; SIADH
Dactinomycin	Antineoplastic antibiotic – DNA intercalation	Wilms	Myelosuppression, hepatopathy
Doxorubicin	DNA intercalation; topo II inhibition	Wilms, CCSK, rhabdoid	Myelosuppression, cardiomyopathy, mucositis, secondary leukemia
Cyclophosphamide	Alkylating agent	Wilms, CCSK, rhabdoid	Myelosuppression, hemorrhagic cystitis, infertility
Etoposide	Topoisomerase II inhibition	Wilms, CCSK, rhabdoid	Myelosuppression, infusion reaction/ hypotension, secondary leukemia
Carboplatin	Alkylating agent	Wilms, rhabdoid	Myelosuppression, hypersensitivity reaction, hearing loss, kidney injury
Irinotecan	Topoisomerase I inhibition	Wilms	Diarrhea, myelosuppression
Ifosfamide	Alkylating agent	Wilms, rhabdoid	Myelosuppression, hemorrhagic cystitis, infertility
Topotecan	Topoisomerase I inhibition	Wilms	Myelosuppresion, rash
Sunitinib, Axitinib, Cabozantinib	Anti-angiogenic	Renal cell carcinoma	Hypertension, rash, proteinuria

10.8 Gy flank RT for DAWT stage I–II and 19.8 Gy for DAWT stage III–IV. Same consideration for whole abdomen RT to 10.5 Gy should be given in DAWT, with boost required in this histology [86]. Nonetheless, debate exists whether all patients with stage 1 diffuse anaplastic WT require radiotherapy [87, 102].

Whole lung radiotherapy has been established as standard of care in the treatment of WT patients with pulmonary metastases. AREN0533 omitted whole lung radiotherapy in patients with lung metastases for patients who met the following criteria: favorable histology WT, no LoH1p/16q, lung only metastases, and a complete response (CR) in the lungs by week 6 interval disease assessment. The 4-year EFS in CR patients with RT omitted and incomplete responding patients (with RT) was 79.5% and 88.5%, respectively. These numbers were superior to prior studies with omission of whole lung irradiation deemed appropriate in the selected group given known toxicities. Whole lung radiotherapy dosing is 12 Gy in 8 fractions for patients above 12 months of age and 10.5 Gy in 7 fractions for those under 12 months of age [89].

Whole liver radiotherapy is advised for patients with liver metastases, typically dosed to 19.8 Gy in 11 fractions with consideration of focal boost for residual tumor at time of radiotherapy. Consideration can be given to resection or focal radiotherapy alone for cases of solitary liver metastases.

Cardiac-sparring WLI techniques as well as IMRT for whole liver RT have demonstrated both feasibility as well as dosimetric advantages over classic 3D conformal techniques [103].

With the increasing use of nephron-sparring resection, particularly in cases of bilateral Wilms tumor or in patients with predisposing conditions for renal disease, focal radiotherapy techniques have also grown in consideration. This may be an option in cases of positive margin, following resection where classically whole flank RT would be used.

Radiotherapy is also considered standard for stage 2–4 CCSK, stage 1–4 RTK, and may have palliative benefit for managing symptomatic loci of pediatric RCC.

Survivorship

With over 90% of children treated for Wilms tumor surviving their disease, as well as improved cure rates for children with CCSK and low stages of other pediatric neoplasia, addressing late effects of therapy related to surgery, radiation, and chemotherapy is a critical healthcare initiative. The Childhood Cancer Survivor Study (CCSS) reported 25-year follow-up of WT survivors, demonstrating the cumulative incidence of all and severe chronic health conditions impacted two-thirds and one-fourth of WT survivors, respectively. Thankfully, mental health status, socioeconomic outcome, and healthcare utilization were similar for WT survivors compared to sibling controls. Radiation exposure to the lungs/chest increased risks for congestive heart failure, especially in patients receiving cumulative doxorubicin in excess of 250 mg/m^2 in total. Overall, considering cardiac concerns and secondary malignancies (slightly increased), overall mortality was marginally increased in pediatric WT survivors treated in the 1970s and 1980s on longer-term follow-up [104].

Site-specific morbidity is also prevalent in survivors, with radiation associated with increased risk for musculoskeletal development (scoliosis and other), gonadal function including fertility (magnified by gonadal toxic therapies such as cyclophosphamide), lung function (radiation pneumonitis), and cardiac insufficiency [105].

Secondary malignancies, not uncommon in chemotherapy-treated children, have been found in Wilms tumor survivors with an absolute excess risk of 7.6/10,000 persons/year, rates substantially lower than most other pediatric cancer survivor populations [106]. One single institution study demonstrated that some WT survivors have increased neurocognitive challenges including poor memory, attention, and general quality of life, independent of treatment variables [107]. Advances in the management of pediatric renal tumors continue to emphasize maximizing survival for higher risk patients and minimization of late effects in lower risk populations [98].

Renal function of survivors has been explored by a number of groups. The SIOP group reported that survivors of non-syndromic unilateral WT have a slightly increased rate of chronic kidney disease after two decades [108]. A report from the St Jude Lifetime Cohort Study reported that no survivor of non-syndromic unilateral WT had an estimated glomerular filtration rate <60 mL/min/1.73 m^2. Hypertension was somewhat common, however, with 35% of irradiated and 25% of unirradiated survivors impacted, compared with 0% controls [109]. Further investigation demonstrated that patients treated with nephrectomy alone, without chemotherapy or radiotherapy, had less impaired renal function with lower rates of proteinuria (12.5%), GFR <90 (21.3%; all >60 mL/min/1.73 m^2) hypertension (6.7%) compared with chemotherapy and/or radiation-treated patients [110].

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Onconephrology

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Abstract

Pediatric oncology patients are at risk for both acute and chronic kidney injury, not only from the cancer itself, but often related to its treatment. Chemotherapy, radiation, medications, infections, hematopoietic stem cell transplant, and newer therapies like CAR-T cells and immune checkpoint inhibitors are beneficial in treating this at-risk population, but also contribute to the risk of kidney injury. Treatment advances in oncology have markedly improved outcomes, with these advances leading to a greater incidence and prevalence of kidney disease in cancer patients. This chapter addresses major nephrology issues affecting the pediatric cancer patient, focusing on acute and chronic management of kidney dysfunction. Within these realms, specific attention is given to kidney injury due to (1) primary oncologic disease, (2) established and emerging treatments, and (3) hematopoietic stem cell transplant. This chapter provides a foundation for the approach to patients that are within the realm of onconephrology, an evolving and expanding field.

Keywords

Cancer · Oncology · Acute Kidney Injury · Chemotherapy · Nephrotoxicity · Hematopoietic Cell Transplant (HCT) · Tumor Lysis Syndrome (TLS) · Sinusoidal Obstruction Syndrome (SOS) · Graft versus Host Disease (GVHD) · Cancer Suvivorship · Chronic Kidney Disease (CKD)

Introduction

Cancer is the second leading cause of death in the pediatric population, with leukemia and brain tumors being the two most common malignancies. Recent treatment advances have led to significant improvements in survival. However, the severity of the underlying malignancy and the use of increasingly aggressive therapeutic regimens can cause organ toxicity with both short-term and long-term consequences. Accordingly, acute kidney injury (AKI) and chronic kidney disease (CKD) can occur in children with cancer and is the result of multiple mechanisms. AKI is estimated to affect approximately half of all pediatric patients with cancer or following hematopoietic stem cell transplantation (HSCT) [1]. AKI and CKD in children with cancer are often multifactorial, with kidney injury from both the disease process itself, as well as the treatments employed to eradicate the disease. This chapter will review the specific causes of AKI and CKD in children with cancer and/or those undergoing HSCT and outline the general approach to the evaluation, prevention, and treatment of kidney disease in this high-risk population. Specific attention will be given to therapy-associated nephrotoxicity (chemotherapy, radiation-induced injury, and newer T cell-based therapies (CAR-T), tumor lysis syndrome (TLS), mechanisms of AKI and CKD in HSCT recipients, and considerations for acute and maintenance renal replacement therapy in children with a history of cancer.

Nephrology Evaluation of the Oncologic Patient

The pediatric patient with cancer may present to the nephrologist at any time during their clinical course. Ideally, those with preexisting kidney disease or prior episodes of acute kidney injury (AKI) are either already known to the Nephrology service, or Nephrology is involved early in their diagnosis and treatment course. Close communication between Oncology and Nephrology is essential to provide a collaborative approach in the evaluation, prevention, and management of kidney disease. A thorough history and physical examination with a particular focus on risk factors for preexisting kidney disease, family history of kidney disease, dialysis, or transplants, and a review of systems elucidating renal and urinary symptoms (edema, dysuria, nocturia, enuresis, and hematuria) should be performed. Also, a review of the patient's oncologic diagnosis, medications, and past and planned treatment courses is essential to understanding risk factors for the development of AKI and/or chronic kidney disease (CKD).

With regard to laboratory studies, a baseline evaluation of glomerular filtration rate (GFR) is standard to determine the risk of AKI and CKD and to accurately dose medications. It should be noted equations that use serum creatinine to estimate GFR (eGFR) may have significant limitations in the pediatric cancer population leading to overestimation of GFR in patients with decreased muscle mass from chronic steroid exposure, suboptimal nutrition, or prolonged hospitalizations leading to deconditioning. Therefore, alternative methods for estimating or formally measuring GFR, independent of muscle mass, have increasingly been used to either replace or supplement creatinine-based eGFR. Serum cystatin C is perhaps the most feasible option for estimating GFR in such situations, and is now widely available in many laboratories either in-house or as a send-out. Cystatin C is a small protein produced by all nucleated cells and is cleared by glomerular filtration and is not significantly affected by changes in muscle mass. Cystatin C has been validated in several populations, and when used in concert with creatinine, helps to provide a more accurate estimation of kidney function [2, 3]. In cases where creatinine and cystatin C give widely discrepant results, it may be necessary to consider formal measurement of GFR using nuclear medicine isotopes or iohexol [4].

Evaluation of the patient's urine, specifically focusing on the degree of hematuria and proteinuria/albuminuria, can provide a baseline from which to compare subsequent studies and estimate the risk of later CKD. Similarly, attention should be paid to the serum electrolytes, especially calcium, magnesium, and phosphate which can be markers of tubular injury from nephrotoxic medications.

In addition to routine electrolytes, uric acid and lactate dehydrogenase (LDH) values can estimate the risk of tumor lysis syndrome (TLS) before starting chemotherapy. After starting therapy or HSCT, the complete blood count (focus on hemoglobin and platelet count), LDH, haptoglobin, and peripheral smear for schistocytes can help to monitor for and diagnose complications from endothelial injury such as thrombotic microangiopathy (TMA).

Hypertension in cancer patients is very common, affecting at least one-third of patients with malignancy or after HSCT [5]. Causes of hypertension include pain, fluid and salt overload, steroids, calcineurin inhibitors, TMA, and tumor infiltration or compression of the kidney or its vasculature. Severe, acute elevations in blood pressure are a risk factor for posterior reversible encephalopathy syndrome (PRES) and seizures [6]. Chronic hypertension can result from prior chemotherapy, radiation, or TMA [7]. Therefore, is it critical to pay close attention to the accurate measurement of blood pressure, both in the outpatient setting and when patients are admitted to the hospital. Auscultatory methods to measure blood pressure are performed both at the onset of care and at each follow-up nephrology visit. Should there be any question of masked hypertension, white-coat hypertension, or difficulty assessing suspected hypertension, the use of 24-hour ambulatory blood pressure monitoring (ABPM) is now recommended as standard of care. Recent studies in both general and pediatric HSCT populations demonstrated the utility of screening APBMs in diagnosing hypertension, masked hypertension, and pre-hypertension [8].

Baseline imaging studies evaluating the kidneys and bladder structure are often helpful in patients with concern for AKI or CKD after cancer diagnosis. Renal ultrasound and more detailed examinations including CT and MRI can assess for tumor involvement of the kidneys or bladder, screen for preexisting congenital anomalies of the kidney or urinary tract, and can detect urinary obstruction or hydronephrosis necessitating urology consultation. Imaging studies such as voiding cystourethrogram (VCUG) or contrast-ultrasonography can be employed when there is concern for voiding dysfunction or obstruction. Chronically, children with a history of cancer may be at higher risk for short- or long-term kidney and bladder abnormalities including transient change in renal size [9], nocturnal enuresis from bladder injury, or renal concentrating defects and renal cysts that require long-term follow-up.

Finally, in certain situations, kidney biopsy can provide helpful information in the diagnosis and management of AKI and CKD in children with cancer. Specific situations where kidney biopsy may be warranted include AKI of unclear etiology, persistent isolated proteinuria or nephrotic syndrome, chronic unexplained decreases in GFR, or when there is concern for TMA or BK polyomavirus (BKPyV) nephropathy, as a renal biopsy in these situations can provide the necessary evidence for an accurate diagnosis [10]. While generally safer than an open biopsy, there are still risks for bleeding with percutaneous biopsy, especially in the cancer patient with thrombocytopenia or uncontrolled hypertension. [11] Therefore, careful planning and consideration is critical in balancing the risks and benefits of biopsies in this patient population.

Acute Kidney Injury

AKI is characterized by an abrupt reduction in GFR, either with or without reductions in urine output, and is a very common complication in cancer patients. A retrospective study demonstrated that greater than 50% of pediatric patients with cancer developed AKI, with an average of nearly two AKI episodes per patient [1]. Similarly, up to 40% of HSCT recipients have also been shown to develop AKI [12], although an individual patient’s risk for AKI after cancer diagnosis or HSCT is highly variable and dependent on relevant co-morbidities, primary cancer diagnosis, and the treatment course. There are many classification systems to define AKI. Currently, most prefer to use the Kidney Disease: Improving Global Outcomes (KDIGO) methodology to define AKI as an increase in serum creatinine over set periods of time and/or reductions in urine output to determine staging of AKI. Other groups have defined AKI using the Pediatric Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (pRIFLE) system or the AKIN criteria [13]. Additionally, the renal angina index (RAI) incorporates a history of HSCT into its grading system [14]. In this section, we first review the broad categories of AKI (pre-renal, intrinsic, and post-renal) that affect the pediatric cancer population and then focus on specific etiologies pertinent to patients with cancer or HSCT recipients including specific nephrotoxic medications and complications.

AKI Due to Hypoperfusion

Ineffective circulating blood volume leading to AKI can manifest in several ways in the pediatric patient with cancer. Volume depletion or dehydration is commonly caused by nausea, vomiting, diarrhea, or other insensible losses. Anorexia or mucositis can lead to marked reductions in oral intake. Therefore, use of enteral feeding tubes, intravenous fluids, or parenteral nutrition is common in patients with cancer. Even with adequate fluid intake, inadequate effective arterial blood volume can be caused by sepsis, effusions, ascites, and heart failure. Liver dysfunction can lead to sinusoidal obstruction syndrome (SOS, described below) and physiologic derangements consistent with hepatorenal syndrome. Capillary leak syndrome can occur due to sepsis or cellular therapies such as chimeric antigen receptor t-cell therapy (CAR-T). Use and choice of fluid intake should be carefully considered in children with cancer. Attention to the patient's intake, output, fluid balance, and daily weight is essential to prevent fluid overload and need for more intensive management. Even in cases of euvolemia and effective arterial volume, abdominal compartment syndrome from tumor burden can result in inadequate perfusion to the kidneys and use of calcineurin inhibitors can result in afferent arteriole vasoconstriction and reduction in glomerular blood flow.

Intrinsic AKI

The glomerular, tubular, vascular, and interstitial components of the kidney can be commonly injured in children with cancer. TMA is defined as endothelial cell injury with microvasculature and laboratory findings notable for hemolytic anemia with schistocytes and thrombocytopenia in the glomerular and proximal peritubular capillaries leading to reductions in kidney function. TMA is likely due to multiple etiologies, including primary disease, chemotherapy and radiation, and infections. Glomerular injury can also occur with medications. Acute tubular necrosis can be caused by shock, sepsis, and drug toxicity. TLS, cast nephropathy, and drug crystal deposition can all cause tubular obstruction. Additionally, drug-induced tubular dysfunction can lead to significant electrolyte derangements. Lymphoma and leukemia can infiltrate the interstitium. Drug-induced acute interstitial nephritis can be caused by a wide range of medications and immunotherapeutics. From an infectious standpoint, both BKPyV and adenovirus can cause nephropathy in HSCT recipients.

Obstruction-Induced AKI

Obstruction can occur from tumor burden and invasion, retroperitoneal fibrosis secondary to radiation, ureteral stenosis secondary to BKPyV or adenoviral infection, nephrolithiasis, or from blood clots secondary to hemorrhagic cystitis after HSCT. Reduced urine output should be evaluated promptly, examining all causes of AKI, with particular attention to volume status. If adequate volume status is demonstrated, a careful trial of diuretics (e.g., a loop diuretic such as furosemide or bumetanide) may be considered as a "furosemide stress test" to assess the patient's ability to make urine [15]. With persistent oliguria or anuria, further evaluation including imaging (either point-of-care or standard ultrasound), laboratory studies, and consultation with urology should promptly be performed to determine need for further intervention to relieve any identified obstruction.

Medication-Induced AKI

Chemotherapeutics are, by design, meant to cause cell toxicity, and can therefore have significant off-target effects throughout the body. Given the high proportion of cardiac output to the kidneys and their roles in toxin and metabolite clearance, there is a high risk for nephrotoxicity after exposure to medications or their metabolites. Such exposure can result in kidney injury, which can be exacerbated in situations of underlying AKI or CKD [16] (Table 3).

Glomerular Injury from Chemotherapy

Glomerular injury due to chemotherapeutics can result in TMA or disruption of the normal glomerular filtration barrier leading to proteinuria. Cessation of the offending agent is often the best treatment for glomerular injury in these situations, but progression to CKD can occur. Mitomycin is an alkylating agent that has been described to cause significant renal dysfunction by TMA-dependent and TMA-independent mechanisms [17, 18], typically in a dose-dependent manner with doses greater than 70 mg/m^2. Therefore, in treatment regimens requiring its use, efforts are made to limit doses to less than 40 mg/m^2 [19]. Gemcitabine is a pyrimidine antimetabolite that is structurally similar to cytarabine, and both cause proteinuria and nephrotic syndrome, which is reversible with cessation of the medication. Gemcitabine also results in TMA in as many as 2.2% of patients. [20]

Antiangiogenic medications targeting vascular endothelial growth factor (VEGF) signaling pathways are increasingly used in the treatment of malignancies, especially in solid tumors where angiogenesis is essential for tumor growth and establishment of metastases. Bevacizumab is a humanized monoclonal antibody that serves as a potent VEGF inhibitor by binding directly to VEGF. Bevacizumab can cause TMA and has also been shown to lead to hypertension, proteinuria/nephrotic syndrome, focal proliferative cryoglobulinemia, and immune-complex mediated proliferative glomerulonephritis [21, 22]. Other antiangiogenic agents include the multi-target tyrosine kinase inhibitors (mTKIs) sunitinib, sorafenib, and pazopanib. These are small molecule inhibitors targeting downstream kinase pathways of VEGF signaling [23]. Typically, renal injury in mTKIs is demonstrated by proteinuria, hypertension, and nephrotic syndrome, with renal biopsy findings demonstrating minimal change disease or focal segmental glomerulosclerosis (FSGS). TMA is also less commonly seen with this class of medications. Notably, there is a case report of thrombotic thrombocytopenia (TTP) associated with pazopanib use that was successfully treated with pazopanib cessation and plasmapheresis, challenging the classic understanding that TTP is not a drug-induced disease [24]. Progression of renal injury with VEGF inhibitors and mTKIs is usually halted or reversed with cessation of the offending agent, and there is some evidence that concomitant use of renin-angiotensin blockade may improve overall patient survival [25], although long-term studies are needed to better understand their impact on the development of CKD.

Tubular Injury from Chemotherapy

Cyclophosphamide and ifosfamide are alkylating agents that serve to disrupt and inhibit DNA synthesis. They are effective in a wide range of malignancies including solid tumors, lymphoma and leukemia, and are also utilized in conditioning regiments for HSCT. Each of these medications causes distinct injury due to toxic metabolites. Cyclophosphamide is metabolized in the liver to generate acrolein, which is an irritant to the genitourinary tract and results in early onset hemorrhagic cystitis after HSCT. Standard prophylaxis includes hyperhydration and administration of mesna, which directly binds acrolein [16]. Ifosfamide is metabolized to chloracetaldehyde, which results in acute tubular necrosis and development of Fanconi syndrome [26]. Higher doses of ifosfamide are associated with a greater degree of CKD [27].

Methotrexate inhibits dihydrofolate reductase, which results in an inability to effectively perform DNA synthesis and cell replication. Methotrexate is widely used in oncology as both chemotherapy and graft-versus-host-disease (GVHD) prophylaxis after HSCT. Methotrexate is both filtered in the glomerulus and secreted into the tubules, and is poorly soluble in acidic urine due to it being a weak organic acid. Typically, low dose methotrexate does not cause any significant renal injury, but at doses greater than 1 g/m^2 it can lead to tubular crystal nephropathy [28, 29]. The resulting AKI can be either oliguric or non-oliguric, depending on the severity of injury, and the urinalysis is generally bland without proteinuria. The risk of methotrexate crystal nephropathy can be reduced by aggressive hydration and urinary alkalinization. Several drugs, including salicylates, penicillin, NSAIDs, and probenecid, are known to interfere with the tubular secretion of methotrexate and increase the risk of methotrexate nephrotoxicity. Treatment of methotrexate nephropathy includes the use of leucovorin which bypasses dihydrofolate reductase inhibition. In severe cases, glucarpidase (also known as CPDG2), which is a recombinant enzyme of bacterial origin that metabolizes methotrexate into inactive compounds, can be utilized as a rescue from significant methotrexate-induced nephropathy [26]. Due to intracellular methotrexate accumulation and also being highly bound to plasma proteins, hemodialysis and hemoperfusion can provide modest overall reductions in methotrexate, but there is significant rebound of methotrexate levels in the blood, typically requiring multiple sessions to achieve sustained reductions in toxic levels of methotrexate [30, 31].

Cisplatin is a platinum-containing chemotherapeutic that induces production of reactive oxygen species and inhibits antioxidant enzyme activity. Cisplatin induces kidney-specific expression of tumor necrosis factor alpha, resulting in programmed cell death. [32]. Organic cation transporter 2 is responsible for luminal uptake and concentration of cisplatin in the renal tubular cells. There is an acute reduction in renal blood flow and associated toxicity within hours of administration, and this effect is dose dependent. The resulting tubular injury causes sodium, phosphate, and magnesium wasting [26]. Histologically, cisplatin injury is characterized by tubulointerstitial injury with acute tubular necrosis, cyst formation, and interstitial fibrosis [32]. Recovery usually follows cessation of cisplatin administration, but magnesium wasting can persist for longer. Careful dose selection and maintaining hydration are the mainstays of minimizing cisplatin toxicity and magnesium supplementation may provide benefit although this is not well validated [33]. Amifostine can serve as an antioxidant to limit cisplatin's nephrotoxicity, but its use is limited due to cost, side effects, and concern for amifostine reducing cisplatin's antitumor potency. Both carboplatin and oxaliplatin are less nephrotoxic [34, 35].

Finally, immunomodulatory therapies can also lead to tubular injury. Ipilimumab is within the family of checkpoint inhibitors that targets CTLA-4 to reduce inhibition of cytotoxic T cells, thereby potentiating the cytotoxic effects of the native immune systems in treating cancer. Both ipilimumab and nivolumab have been associated with AKI [36], granulomatous allergic interstitial nephritis [37], and a case report of lupus nephritis [38]. With more clinical interest and research focusing immunomodulation and checkpoint inhibitors, both the use of these drugs, and associated kidney injury, may increase with time. Furthermore, cell-based therapies such as CAR-T are a novel and powerful tool to treat refractory cancers, but have been reported to cause AKI [39–41]. In CAR-T cell therapy targeting leukemia, studies have shown significant risk for AKI associated with an inflammatory state called cytokine release syndrome that results in multiorgan dysfunction [42, 43]. As this therapeutic approach is in the relatively earlier stages of use, more data is needed to better understand the mechanisms of kidney injury.

Tumor Burden and Resection

Intraparenchymal renal tumors are relatively uncommon in the pediatric population, with Wilms tumor being the most common pediatric renal tumor. Germline *WT1* mutations are responsible for a variety of associated syndromes such as Wilms tumor-aniridia syndrome (WAGR), Denys-Drash syndrome (DDS), and Frasier syndrome [44], all which have an increased risk for developing Wilms tumor [44]. In contrast to the adult population, pediatric presentation of renal cell carcinoma is much less prevalent [16]. Leukemia, lymphoma, and neuroblastoma can infiltrate renal parenchyma and cause significant direct injury, hypertension, and obstruction [16]. Extrarenal tumors such as bladder rhabdomyosarcomas can also obstruct the urinary tract. For unilateral renal tumors, surgical nephrectomy is the mainstay of treatment. Often, there is adequate preserved renal function with compensatory hypertrophy in the solitary kidney, albeit with reduced renal reserve [45]. Patients are prone to further renal injury and the development of hypertension [46, 47]. However, long-term follow-up studies in patients with Wilms tumor treated with unilateral nephrectomy demonstrate hyperfiltration and FSGS [48], but the overall incidence of end-stage kidney disease (ESKD), as defined by dialysis or renal transplant, is low between 1 and 2% [49]. In contrast, Wilms tumor patients requiring bilateral partial nephrectomies demonstrate a higher risk for ESKD [50, 51].

Radiation-Induced Injury

Radiation therapy is used as either a treatment for certain types of malignancies or as part of the conditioning regimen prior to HSCT. Typically, radiation therapy regimens use focused or total body irradiation (TBI), and conditioning regimens utilize TBI [52]. Radiation to the kidney is associated with

Table 1 Dosing Considerations for Cancer Therapeutics in CKD and ESKD. This table is adapted from Krens et al [93]. There are limited data for many chemotherapeutics in CKD and ESKD, especially in the pediatric population. There are several helpful references that can be used to determine optimal dosing and frequency, although there can be significant variation in the recommendations. These resources (listed at the bottom of this table) can and should be utilized in concert with pharmacists and oncologists, in addition to manufacturer-provided prescribing information (e.g., US-FDA clearinghouse https://dailymed.nlm.nih.gov/dailymed/index.cfm)

Cancer treatment medications requiring dose adjustment consideration in CKD or ESKD

Agent	Recommendations	
Arsenic trioxide	GFR $\geq$30 mL/min: no need for dose adjustment is expected GFR <30 mL/min: consider 50% of the original dose HD: consider 10 mg three times weekly post-dialysis	
Bleomycin	GFR >50 mL/min: no dose adjustment is needed GFR 10–50 mL/min:75% of the original dose GFR <10 mL/min: 50% of the original HD: 50% of the original dose may be considered	
Carboplatin	Renal impairment: dose calculation based on renal function, using Marina/St. Jude's formula (pediatric formula adapted from Calvert's formula) HD: dose according to Marina/St. Jude's formula with GFR equals 0. Perform HD between 12 and 24 h after administration ***Marina/St.Jude's formula:*** *Dose* **(mg/m^2)** = *Target AUC x [(0.93 x corrected GFR normalized to patient's BSA (mL/minute/m^2)) +15]*	
Carmustine	GFR 46–60 mL/min: 80% of the original dose GFR 31–45 mL/min: 75% of the original dose GFR <30 mL/min: not recommended HD: not recommended	
Cisplatin	*Curative* GFR 50–59 mL/min: 75% of the original dose GFR 40–49 mL/min: 50% of the original dose GFR <40 mL/min: not recommended HD: 50% of the original dose may be considered	*Palliative* GFR 50–59 mL/min: 75% of the original dose GFR <50 mL/min: not recommended HD: not recommended, consider carboplatin
Cyclophosphamide	GFR $\geq$30 mL/min: no dose adjustment is needed GFR 10–29 mL/min: consider 75% of the original dose GFR <10 mL/min: not recommended, if unavoidable consider 50% of the original dose HD: not recommended, if unavoidable consider 50% of the original dose	
Cytarabine (high-dose $\geq$ 1 g/m^2) for low-dose, no dose adjustments are needed	GFR $\geq$60 mL/min: no dose adjustment is needed GFR 31–59 mL/min: 50% of the original dose GFR <30 mL/min: not recommended HD: 50% of the original dose, start HD 4–5 h after administration	
Dacarbazine	GFR $\geq$30 mL/min without hepatic impairment: no dose adjustment is needed GFR <30 mL/min: 70% of the original dose may be considered HD: 70% of the original dose may be considered	
Daunorubicin	GFR 30–50 mL/min: 75% of the original dose GFR <30 mL/min: 50% of the original dose HD: 50% of the original dose	
Doxorubicin	GFR >10 mL/min: no dose adjustment is needed GFR <10 mL/min: no need for dose adjustment is expected HD: 75% of the original dose may be considered	
Etoposide (Intravenous)	GFR >50 mL/min: no dose adjustment is needed GFR 10–50 mL/min: 75% of the original dose, increase if tolerated HD: not dialyzed, consider 75% of the original dose	

(continued)

Table 1 (continued)

Fludarabine	GFR >70 mL/min: no dose adjustment is needed GFR 30–70 mL/min: 80% of the original dose GFR <30 mL/min: not recommended HD: 80% of the original dose. Start dialysis 12 h after administration
Gemcitabine	GFR ≥30 mL/min: no dose adjustment is needed GFR <30 mL/min: no need for dose adjustment is expected HD: no need for dose adjustment is expected. Start HD 6–12 h after administration
Hydroxycarbamide (Hydroxyurea)	GFR <60 mL/min: 50% of the original dose HD: 50% of the original dose
Ifosfamide	GFR > 10 mL/min: no dose adjustment is needed GFR <10 mL/min: 75% of the original dose HD: 1,000 mg/m^2 followed by HD 6–8 h later
Imatinib	GFR 40–59 mL/min: no need for dose adjustment is expected GFR 20–39 mL/min: 50% of the original dose, increase if tolerated HD: use with extreme caution
Irinotecan	GFR ≥10 mL/min: no need for dose adjustment is expected GFR <10 mL/min: start with 50–66% of the original dose, increase if tolerated HD: start with 50–66% of the original dose, increase if tolerated
Melphalan	*Conditioning treatment*: Renal impairment/HD: no need for dose adjustment is expected *Palliative treatment* GFR 30–50 mL/min: consider 50% of the dose GFR <30 mL/min or HD: not recommended
Mercaptopurine	GFR ≥30 mL/min: no need for dose adjustment is expected, can consider dosing interval to 48 h GFR <30 mL/min: increase dosing interval to 48 h HD: not recommended
Methotrexate	GFR ≥50 mL/min: no dose adjustment is needed GFR 20–50 mL/min: 50% of the original dose GFR <20 mL/min not recommended. If unavoidable consider hemodialysis HD: not recommended, if unavoidable 50% of the original dose, can be dialyzed with daily high flux dialysis or hemofiltration
Mitomycin	GFR ≥30 mL/min: no need for dose adjustment is expected GFR <30 mL/min or HD: not recommended due to nephrotoxicity
Oxaliplatin	GFR ≥30 mL/min: no dose adjustment is needed GFR <30 mL/min: consider 50% of the original dose HD: consider 50% of the original dose, HD within 1–5 h after administration
Pemetrexed	GFR ≥40 mL/min: no dose adjustment is needed GFR 30–40 mL/min: 80% of the original dose GFR <30 mL/min: not recommended HD: not recommended
Sorafenib	GFR >40 mL/min: no dose adjustment is needed GFR 20–39 mL/min: 200 mg BID, dose-escalation based on tolerability GFR <20 mL/min or HD: 200 mg QD, dose-escalation based on tolerability
Streptozocin	GFR 46–60 mL/min: 50% of original dose GFR 31–45 mL/min: not recommended, if unavoidable consider 25% of the original dose GFR ≤30 mL/min or HD: not recommended

(continued)

Table 1 (continued)

Sunitinib	Renal impairment: no dose adjustment is needed HD: no initial dose adjustment is needed, increase dose based on sunitinib + active metabolite C_{trough} levels:
Thiotepa	GFR $\geq$30 mL/min: no need for dose adjustment is expected GFR <30 mL/min: consider 70% of the original dose, increase if tolerated HD: consider 70% of the original dose, increase if tolerated
Topotecan (Oral and intravenous)	GFR $\geq$40 mL/min: no dose adjustment is needed GFR 20–39 mL/min: 50% of original dose GFR <20 mL/min: not recommended, if unavoidable consider 25% of the original dose HD: not recommended, if unavoidable consider 25% of the original dose

Cancer treatment medications not typically requiring dose adjustment for CKD or ESKD

Asparaginase	Dactinomycin	Gemtuzumab-ozogamicin	Ofatumumab	Tamoxifen
Bevacizumab	Dasatinib	Inotuzumab ozogamicin	Paclitaxel	Temozolomide
Blinatumomab	Docetaxel	Irinotecan	Panitumumab	Trastuzumab
Bortezomib	Everolimus	Mitotane	Pegaspargase	Vinblastine sulfate
Busulfan	Fluorouracil	Nivolumab	Pembrolizumab	Vincristine sulfate

Additional Resources for dose adjustment evaluation in CKD or ESKD:
Aronoff GM, Bennett WM, Berns JS, et al. Ref. [144]
Kintzel PE, Dorr RT Ref. [145]
https://www.cancercareontario.ca/en/cancer-treatments/chemotherapy/drug-formulary
https://dailymed.nlm.nih.gov/dailymed/index.cfm

long-term nephrotoxicity [46, 53] causing damage to the glomerulus, tubules, and vascular endothelium [54]. Conflicting data exists regarding the long-term effects of TBI on kidney function, and indeed may be dose-dependent [55] [56, 57], as many of these studies demonstrating delayed renal dysfunction received cumulative prescribed radiation greater than recommended in Children's Oncology Group (COG) guidelines [58].

Tumor Lysis Syndrome

TLS occurs after significant cell lysis of tumor cells and release of their intracellular contents into the bloodstream, leading to hyperkalemia, hyperphosphatemia, hypocalcemia, and hyperuricemia. These electrolyte and metabolite derangements can cause complications in multiple organs including AKI, seizures, and arrhythmias. While TLS can occur spontaneously in patients with Burkitt's lymphoma, it is most commonly encountered shortly after initiation of chemotherapy [52]. Risk factors for TLS include the type of cancer, disease burden at presentation, and the specific treatment regimen. TLS is the most common oncologic emergency with significant risks for morbidity and mortality if not promptly diagnosed and managed (Table 2).

From a pathophysiologic perspective, the rapid increase of released cellular contents into the bloodstream exceeds the normal clearance of the kidney, and the patient with concomitant AKI or CKD is even more susceptible to significant injury. Uric acid, which is a breakdown product of purines, is normally freely filtered in the glomerulus and reabsorption and secretion is regulated by URAT-1 and OAT. With the massive increase of uric acid from highly proliferative tumor cell lysis, there is an accumulation of uric acid crystals within the tubular lumen, which causes direct mechanical injury and obstruction, but has also been demonstrated to cause increased inflammation, increased peritubular capillary pressures, and inhibition of cell repair mechanisms, all of which can contribute to significant AKI. Phosphate, which is a predominantly intracellular electrolyte, is significantly increased in

Table 2 Tumor lysis syndrome definition and risk characterization

A. Cairo-Bishop definition of tumor lysis syndrome (Cairo-Bishop)

Laboratory TLS		**Clinical TLS**
Uric acid	≥8 mg/dL or 25% increase from baseline	Laboratory TLS, **and** ≥1 of the following:
Potassium	≥6 mEq/L or 25% increase from baseline	Creatinine ≥1.5×ULN (for >12 yo)*
Phosphorus	≥6.5 mg/dL (children), ≥4.5 mg/dL (adults), or 25% increase from baseline	Cardiac arrhythmia or sudden death
Calcium	≥25% decrease from baseline	Seizure

*Classic definition limits evaluation in CKD patients and patients <12 yo. Recommend using appropriate definitions for AKI when original schema is inadequate

B. Risk characterization of tumor lysis syndrome

First, determine disease risk by cancer type and relevant characteristics, Then assess relevant modifying factors to determine risk category for recommendations on TLS prophylaxis

Disease risk	**Type of tumor**	**Modifying factors**
Low risk disease	Solid tumors (excluding bulky tumors such as neuroblastoma and germ-cell tumors)	For LRD leukemia and lymphoma in patients with renal dysfunction or renal involvement, consider as intermediate risk for developing TLS
	Myeloma	
	CML	
	CLL treated only with alkylating agents	
	AML w/WBC, <25 × 10^9 and LDH, <2×ULN	
	Lymphoma, except as below	
Intermediate risk disease	Bulky solid tumors (e.g., neuroblastoma and germ-cell tumors)	For IRD in patients with renal dysfunction or renal involvement, consider as high risk for developing TLS
	CLL treated with targeted or biological therapies	
	AML w/WBC, <25 × 10^9/L and LDH, >2×ULN	
	AML w/ WBC 25–100 × 0^9/L	
	ALL w/ WBC <100 × 10^9/L and LDH, <2×ULN	
	Burkitt or lymphoblastic, early stage with LDH<2×ULNL	For IRD in patients with uric acid, phosphate, or potassium above normal limits, consider as high risk for developing TLS
	Lymphoma, Anaplastic large-cell, Stage III/IV, LDH, <2×ULN	
	Lymphoma, Stage III/IV	
	ATL, diffuse large B-cell, peripheral T-cell, mantle cell (blastoid variants)	
High risk disease	AML with WBC>100 × 10^9/L	
	ALL with WBC>100 × 10^9/L	
	ALL with LDH>2×ULNL	
	Lymphoma, advanced stage with LDH>2×ULNL	
	Burkitt or lymphoblastic lymphoma, early stage, with LDH>2×ULNL	

(continued)

Table 2 (continued)

B. Risk characterization of tumor lysis syndrome		
	Burkitt or lymphoblastic lymphoma, advanced stage	
Prophylaxis recommendations based on TLS risk		
Low risk	**Intermediate risk**	**High risk**
Intravenous fluids	Intravenous fluids	Intravenous fluids
Allopurinol	Allopurinol or rasburicase	Rasburicase
Monitoring	Inpatient monitoring	Cardiac monitoring
Daily laboratory tests	Laboratory tests 2–3×/day	Laboratory tests 3–4×/day

Table providing Cairo-Bishop diagnostic criteria for tumor lysis syndrome, as well as summary of relevant pediatric cancer risk characterization and recommended prophylaxis for prevention of tumor lysis syndrome. For more complete risk characterization, including adult and more rare cancers, please refer to Cairo et al. 2010 [61]

the bloodstream with tumor lysis. Hyperphosphatemia leads to phosphate avidly binding to calcium, resulting in hypocalcemia that can cause acute cardiovascular and neuromuscular complications, as well as calcium-phosphate precipitation and deposition in tissue, including the kidney. Hyperkalemia also is common with TLS and can result in significant morbidity and mortality.

Several scoring systems have been proposed to help define and stratify the risk of TLS [59, 60]. The modified Cairo-Bishop definition uses specific changes in electrolytes as laboratory markers of TLS, and the presence of arrhythmias, sudden death, seizures, or a >1.5 times increase in serum creatinine above the upper limit of normal to define TLS. These criteria assume adequate hydration status, and derangements are not due to medications, and prophylaxis with a hypouricemic agent. Further work stratifies the risk of severity for specific presentations of cancer, to allow for prognosis and decision making in management [61], (Table 2). Limitations of the scoring system include that AKI is only defined for adolescents and older children and using 1.5 times the upper limit of normal serum creatinine may underestimate risk in those with CKD or decreased muscle mass.

Management of TLS begins with prophylactic strategies. Nephrotoxic medications should be avoided as possible, and regular labs should be obtained to monitor progression of TLS. Volume expansion and rates of intravenous fluid administration that promote brisk urine output ensure adequate renal perfusion and promote excretion of uric acid, potassium, and phosphate. Alkalinization of the urine does improve the solubility of uric acid, but at the same time promotes precipitation of calcium-phosphate crystals, and therefore is not recommended. Administration of allopurinol or febuxostat, xanthine oxidase inhibitors, help to prevent purine conversion of uric acid, and given their mechanism of action, are more effective as prophylactic rather than treatment measures. Notable differences between allopurinol and febuxostat are that febuxostat does not have the same concerns for hypersensitivity as allopurinol; it is metabolized by the liver, rather than the kidney; it does not need dose adjustments for a reduced GFR; and it has fewer drug interactions with chemotherapy than allopurinol. For treatment of hyperuricemia rasburicase is used, which is a fungal-derived uricase oxidase enzyme that works by converting uric acid to allantoin and is much more soluble than uric acid, thereby decreasing hyperuricemia. Rasburicase can also be used prophylactically in high-risk situations and does not require altered dosing for diminished GFR. The patient's G6PD status should be evaluated prior to use, since rasburicase use in patients with G6PD deficiency can cause methemoglobinemia and hemolysis. It should also be noted that rasburicase's activity persists in blood after collection, so samples should be processed promptly or placed on ice to ensure accurate results. Use of the above preventive measures has greatly reduced the need for renal replacement therapy in TLS patients. Should these more conservative

Table 3 Chemotherapy-induced renal dysfunction

A. Common chemotherapy-induced nephrotoxicity	
Chemotherapeutics with glomerular toxicity	
Agent	**Injury**
Gemcitabine	TMA, hypertension, AKI
Mitomycin	TMA, AKI, hypertension (dose-dependent)
Bevacizumab	TMA, proteinuria/nephrotic syndrome, hypertension
VEGFR mTKI (Sunitinib, Sorafenib, Pazopanib, Nivolumab)	TMA, minimal change disease, (collapsing) FSGS, AKI
Chemotherapeutics with tubular toxicity	
Agent	**Injury**
Cisplatin	ATN AKI, Chronic interstitial fibrosis
Ifosfamide	ATN, Fanconi syndrome
Methotrexate	Crystal nephropathy, nonoliguric AKI
Pemetrexed	ATN AKI AIN, Tubular atrophy and interstitial fibrosis
Ipilimumab	AIN
Chemotherapeutics with genitourinary toxicity	
Agent	**Injury**
Cyclophosphamide	Hemorrhagic cystitis
B. Common chemotherapy-induced electrolyte abnormalities	
Electrolyte abnormality	**Common causative agent**
Hyponatremia	Cisplatin, cyclophosphamide, ifosfamide, methotrexate, pemetrexed, interferons, vincristine, vinblastine
Hypokalemia	Cisplatin, glucocorticoids, ifosfamide
Hypomagnesemia	Cisplatin, calcineurin inhibitors, ifosfamide, EGFR inhibitors (cetuximab and panitumumab)
Hypophosphatemia	Cisplatin, Ifosfamide, and imatinib

Table providing commonly used chemotherapeutics and associated toxicities. (Adapted from Glezerman et al [22]), and electrolyte abnormalities associated with common chemotherapeutic agents

measures prove inadequate and the patient is demonstrating life-threatening symptoms or lab values, the use of either hemodialysis (HD) or continuous renal replacement therapy (CRRT) with high clearance rates is warranted [62], with the risk of interdialytic rebound of serum electrolytes less likely with CRRT.

AKI Associated with HSCT

Sinusoidal Obstruction Syndrome

SOS is another common complication of HSCT associated with AKI. The hepatic sinusoidal endothelial injury that occurs causes significant liver dysfunction and portal hypertension, with resultant systemic hypotension, AKI, and an increased risk for multiorgan dysfunction and mortality. Previously described as veno-occlusive disease (VOD) of the liver, SOS has become the preferred nomenclature, due to a better understanding of the specific hepatic sinusoidal cell injury that occurs. SOS is very common in pediatric HSCT recipients, with estimates of prevalence ranging between 20% and 30%. In children with SOS, severe disease involving multiorgan dysfunction is as high as 30–60%, a rate higher than that seen in the adult population. SOS appears similar to hepatorenal syndrome, with volume overload, ascites, jaundice, and hepatomegaly. Due to portal hypertension, there is systemic hypertension, hyponatremia, and evidence of renal dysfunction (Table 4).

There are multiple risk factors for SOS including age <1-year, preexisting liver dysfunction, prior transplant, prior radiation treatment, and conditioning including busulfan (with or without cyclophosphamide) and fludarabine. Generally, the more intensive the conditioning regimen and the less well-matched the transplant, the higher risk of SOS. SOS typically presents within 30 days of transplant but in the pediatric population it can occur at any time after transplantation. There had classically been two major classification schemata utilized for the diagnosis of SOS, the modified Seattle [63, 64] and the Baltimore [65] criteria, both focusing on hepatomegaly, weight gain, and hyperbilirubinemia in the first three weeks following HSCT. Review of these two classification systems in pediatric cancer patients suggested that they may be too restrictive for accurately diagnosing pediatric SOS [66], with

Table 4 Schemata for diagnosis of sinusoidal obstruction syndrome (SOS)

	Baltimore Criteria	**Modified Seattle Criteria**	**EBMT Criteria-Pediatric**
Timing:	Within 21 days of transplantation	Within 20 days of transplantation	No time limitation after transplantation
Findings:	Serum bilirubin ≥ 2 mg/dL, **AND ≥2 of the following findings**	**≥2 of the following findings**	**≥2 of the following findings**
	Hepatomegaly	Hepatomegaly	Hepatomegaly (best if confirmed by imaging) above baseline
	Weight gain >5% above baseline	Unexplained weight gain > 2% from baseline	Unexplained weight gain on 3 consecutive days, despite the use of diuretics, **OR** a weight gain >5% above baseline
	Ascites	Bilirubin serum level > 2mg/dL	Ascites (best if confirmed by imaging) above baseline
		Right upper quadrant pain	Rising bilirubin from a baseline value on 3 consecutive days, **OR** bilirubin ≥ 2 mg/dL within 72 h
			Unexplained consumptive and transfusion-refractory thrombocytopenia

Table providing sinusoidal obstruction syndrome (SOS) criteria from the Baltimore [65], modified Seattle [63], and the European Society for Bone Marrow Transplantation (EBMT) [67] guidelines. Other causes that could explain these findings should be excluded prior to diagnosis. The EBMT recommendations provide specific diagnostic and severity criteria for both the pediatric and adult population, and the adult criteria are available in the EBMT references provided

diagnosis limited by the short post-transplant time cutoff and absolute lab values more appropriate for adult patients. To address these limitations, the European Society for Bone Marrow Transplantation (EBMT) recently developed distinct adult and pediatric diagnostic criteria, [67], and all three SOS classification schemata listed for comparison in Table 4. When suspected, additional laboratory testing in SOS commonly demonstrates elevated transaminases, hyperbilirubinemia, elevated coagulation parameters, and hypoalbuminemia. Up to one-third of all pediatric SOS patients do not present with hyperbilirubinemia and this is often a finding later in the progression of disease, so normal bilirubin levels do not rule out SOS. Although only part of the EBMT criteria for diagnosis, thrombocytopenia that does not improve with platelet transfusions is often observed early in the disease course, and this refractory thrombocytopenia can precede other diagnostic criteria by a week [68]. There have been attempts to find alternative biomarkers for endothelial injury in SOS including PAI-1, thrombomodulin, VWF, soluble ICAM-1, ATIII, and VCAM. Liver biopsy can be used to definitively diagnose SOS, but the diagnosis is largely a clinical diagnosis, and in the context of clinical presentation, labs, and imaging findings, a diagnosis can be made without risking the complications of a liver biopsy.

With the concern for significant morbidity and mortality in SOS, careful monitoring, prophylaxis, and prompt treatment is essential to minimize risk of progression to multiorgan dysfunction and death. Ursodiol is utilized as a prophylactic treatment for SOS, with demonstration of improvement in mortality with its use. Fluid management of SOS remains challenging due to the portal hypertension and resultant systemic hypotension. Adequate systemic intravascular volume needs to be maintained to ensure adequate organ perfusion and can be achieved with resuscitative and maintenance intravenous fluid, but care and attention should also be taken to avoid volume overload in these patients.

Defibrotide, a preparation of oligo-deoxyribonucleotides derived from porcine intestine, is the only medication currently approved for the treatment of SOS and has been shown to provide a survival benefit. The precise mechanisms of action for defibrotide remain to be elucidated, but it demonstrates significant anti-inflammatory and anti-thrombotic effects. Defibrotide is not renally cleared and as such does not require renal dosing for impairments in renal function. Studies in Europe have also demonstrated that prophylactic use of defibrotide has provided benefit in reducing the rate of SOS, but additional studies are still ongoing, and its use is not widely employed as a preventative intervention.

Thrombotic Microangiopathy

There are multiple causes for TMA in children with cancer including chemotherapy, medications, hypertension, and secondary to HSCT. TMA is most commonly diagnosed after HSCT and is associated with higher post-transplant mortality as severe cases can be associated with multisystem organ dysfunction involving the lungs, gastrointestinal tract, and central nervous system [69]. TMA presenting after HSCT may be caused by radiation, or specific agents such as VEGF inhibitors (bevacizumab) and antimetabolites (gemcitabine), as described above [26]. Calcineurin inhibitors in conjunction with sirolimus may also increase the risk of TMA after HSCT [70, 71]. Other risk factors include acute GVHD, infection, and type of transplant [69, 72], and there has been evidence that TMA severity is worsened by complement pathway mutations present in either the recipient, or the HSCT itself [73, 74]. TMA is a microvascular disease than can lead to activation and recruitment of inflammatory cells and activation of coagulation and complement pathways [72]. The resulting damage to the kidney can lead to hypertension, AKI, and proteinuria [26]. Further clinical indicators of TMA include thrombocytopenia or increased platelet transfusion requirements, anemia or increased red cell transfusion requirements, elevated LDH, low haptoglobin, and schistocytosis. Notably, hypertension may be an early clinical indicator of TMA, with a common characteristic of the patient requiring additional antihypertensive agents beyond that expected for the current clinical course, with the requirement for three antihypertensives greatly raising the concern for TMA [69]. TMA most commonly presents at 5–7 weeks following HSCT with most cases presenting before day 100. TMA in autologous recipients occurs even earlier, with a median of 18 days following HSCT [75]. Renal biopsy in TMA demonstrates thrombi within glomeruli and small arterioles, mesangiolysis, and proliferative or sclerotic lesions [76]. Increased LDH and proteinuria have also been shown to be early markers for TMA, while changes in serum creatinine require significant renal injury [69]. Thrombotic thrombocytopenic purpura, a TMA typically due to antibody-mediated inhibition of ADAMTS13, is uncommon but reported in the HSCT literature and should be ruled out by ADAMTS13 testing, if clinically suspected [77]. While CNI withdrawal can be considered if no other cause is found, it is essential that a careful evaluation for infection, GVHD, or other etiology should be performed first. Other immunosuppressive approaches include transition to steroids or mycophenolate as alternative immunosuppressive agents, prompt use of plasma exchange, rituximab, and eculizumab [72], although to date no randomized clinical trials have been conducted with these interventions in the treatment of TMA after HSCT.

Graft-Versus-Host Disease

GVHD occurs with allogeneic HSCT and is characterized by injury to native host tissue, particularly epithelial damage from immune responses from the allograft, and mediated by donor T-cell and cytokine effects. Acute GVHD primarily targets the skin, liver, and gastrointestinal tract and is a risk factor for AKI. Acute and chronic GVHD may also involve the kidney, either through direct T cell-mediated injury or chronic systemic inflammation leading to renal endothelial and tubular damage [72]. Kidney biopsies performed in patients with acute GVHD classically demonstrate tubulitis and peritubular capillaritis, as well as complement deposition and pathologic findings of TMA [76]. Elafin, a marker of skin GVHD, has also been studied in HSCT patients with kidney injury, and higher levels of elafin and associated with persistent albuminuria and progression to CKD [78]. A diagnostic clue for evaluating risk of GVHD is review of the chimerism of the donor T cells. If there is mixed chimerism, it suggests tolerance and reduced risk of GVHD, while full donor T-cell chimerism may indicate that tolerance has been broken and the patient is at risk for GVHD [79]. Progression of disease to chronic GVHD can be associated with significant glomerular injury and development of minimal change disease and membranous nephropathy on histology. Kidney biopsy may provide diagnostic guidance when considering GVHD, as changes in immunosuppression regimens need to be carefully considered in these patients.

Proteinuria and Nephrotic Syndrome

Proteinuria is common among the cancer and HSCT population, with reports of proteinuria being observed in more than a third of HSCT patients with AKI [80]. Proteinuria has also been demonstrated to be helpful in assessing the prognosis of TMA in HSCT patients [69], and albuminuria at 100 days following HSCT is associated with significantly greater risks of CKD and non-relapse mortality [81].

Nephrotic syndrome can present after HSCT and typically occurs >100 days after transplant. Nephrotic syndrome can occur with or without AKI, and there appears to be a greater risk for those who had developed GVHD, and nephrotic syndrome typically presents with reductions in intensity of immunosuppression [72]. Biopsy results demonstrate mainly membranous nephropathy or minimal change disease, but membranoproliferative glomerulonephritis, FSGS, and IgA nephropathy have also been reported. Given the wide range of etiologies observed, the use of a renal biopsy should be considered in HSCT recipients with nephrotic range proteinuria to help guide immunosuppressive treatment, especially if renal findings are associated with acute or chronic GVHD [26]. Rituxumab has also demonstrated efficacy in treatment for membranous-associated nephrotic syndrome [82].

Dialysis in AKI

Studies have suggested that approximately 1–2% of all patients with cancer, and up to 5–10% of pediatric HSCT recipients will require acute RRT [41, 83, 84, 85]. Mortality in children with cancer requiring CRRT has been reported to be greater than 50% [86], and the 1 year mortality rate for HSCT recipients requiring RRT is nearly triple those not requiring dialysis (85% vs. 29%) [84]. The indications for RRT in children with cancer are the same as those in the general population, including refractory volume overload, refractory metabolic acidosis and electrolyte disturbances, symptomatic uremia, and intoxication with dialyzable medications. Despite this overlap in the indications for dialysis, patients with cancer are disproportionately more likely to require RRT owing to the severity of their disease and complications from therapies, which is evidenced by the tripling of the likelihood of mortality in CRRT patients, when they have a diagnosis of cancer [87].

The choice of acute RRT modality is determined primarily by the patient's presentation and level of acuity but is also influenced by physician and institution preferences. Typically, the choices include intermittent HD, CRRT, and peritoneal dialysis (PD). All three modalities have unique benefits and challenges. In the critically ill patient, the high blood flow rate and significant fluid shifts that occur during HD limit its use, favoring the use of CRRT and PD in the ICU. Given the rapid availability of central venous access in the intensive care setting, CRRT is commonly used in resource-rich settings, but PD has also been used as a mode of RRT in the critically ill patient, and with careful management, dialysis can be initiated nearly immediately after PD catheter placement. One major benefit of CRRT is the ability to continuously control relative contributions of dialytic and convective clearance, while providing the ability to titrate ultrafiltration by the bedside nurse to achieve the degree of volume removal necessary to optimally support the patient [88]. This slow and sustained fluid removal in hemodynamically unstable patents makes it the often-preferred modality of RRT in the intensive care setting when all three modalities are available. The intermittent filling and emptying typical with PD makes it a slightly less preferred modality, but PD's utility in critically ill patients has been repeatedly demonstrated [89], and is a reasonable RRT modality when vascular access limitations do not allow for central venous access. For clinically stable patients, HD provides a rapid and efficient means to provide RRT over several hours and therefore allows patients to participate in rehabilitation, therapy, education, play, or other developmentally appropriate activities.

Drug Dosing in CKD and ESKD

Accurate dosing of medications, especially chemotherapy, is critical in patients with AKI and requires close collaboration with inpatient pharmacy teams. Table 1 provides recommendations regarding dosing in CKD and ESKD for more common oncologic medications, but it is beyond the scope of this chapter to provide specific guidance for all medications in cancer patients with reduced kidney function. However, there are several considerations that help to best approach balancing effective treatment with minimizing drug toxicity and harmful side effects. Of principal importance is shared management with oncology, nephrology, and pharmacy. Review of the available literature can be used to determine optimal dosing for patients on CRRT, and the specific expertise of pharmacists is particularly useful, especially given that dosing decisions need to be made based on limited available evidence. Common strategies include dose adjustments, less frequent dosing intervals, and allowing laboratory data such as drug levels, markers of renal and hepatic function, and, as in the case of marrow suppression, blood cell counts [90]. Additionally, there are guidelines for many medications that provide thresholds for reducing the dose of or holding medications, which often are guided by eGFR. With regard specifically to dosing while on dialysis, specific guidelines exist for many chemotherapies, with guidelines for timing and frequency of administration relative to dialysis [91, 92] [93].

Electrolyte and Volume Disturbances

Hyponatremia

Hyponatremia is the most common electrolyte disturbance observed in cancer patients and has been demonstrated to have negative correlations with outcomes [94]. There are multiple causes for hyponatremia including paraneoplastic syndromes resulting in antidiuretic hormone (ADH) production, side effects from chemotherapeutic and adjunctive medications, concomitant medical conditions, and iatrogenic causes including total parenteral nutrition and intravenous fluid administration [94]. Although more common in the adult population, paraproteinemia can be a cause of pseudohyponatremia, that should be considered in the appropriate clinical context. Multiple chemotherapeutics can directly cause hyponatremia via syndrome of inappropriate diuretic hormone release (SIADH), including cyclophosphamide, platinum alkylating agents, methotrexate, interferons, vincristine, vinblastine, and ifosfamide. Cyclophosphamide and cisplatin also cause hyponatremia by increasing tubular ADH efficacy and/or direct tubular injury preventing sodium reabsorption [95]. Hyponatremia can also be induced as a side effects of chemotherapeutics, including nausea, vomiting, cardiac toxicity from doxorubicin and other anthracyclines, and endocrine disruption from immunotherapeutics. Mass lesions from brain cancer can also result in sodium derangements. Medications associated with the management of cancer complications are also responsible for hyponatremia, with SIADH being the etiology for opioid, antidepressant, antipsychotic, and antiepileptic-mediated hyponatremia, and increased ADH efficacy in the nephron as the cause for NSAID and antiepileptics-mediated hyponatremia [96]. As with all management of perturbations of dysnatremia and ADH signaling, evaluation of volume status, weight, and urine and plasma osmolality are essential in determining the optimal course of treatment, which includes careful fluid administration, fluid restriction when necessary, and consideration of vaptans and demeclocycline. The chronicity of hyponatremia should be assessed, and in accordance with guidelines for any patient with sodium derangements, excessively rapid correction should be avoided.

Hyperkalemia

The common causes of hyperkalemia in any patient also apply to patients with cancer. Additionally, evaluation of cell lytic processes such as rhabdomyolysis, TLS, metabolic acidosis, underlying or drug induced diabetes, and medications should be performed. With the prevalence of leukocytosis and thrombocytosis in select populations, excessive lysis of cells within the laboratory sample tube after collection should be assessed to exclude pseudohyperkalemia. Given the risk and incidence of AKI and CKD in cancer patients, careful assessment of potassium intake, through nutrition, supplementation, and medications, should be closely monitored, to avoid iatrogenic hyperkalemia. Hyperkalemia secondary to TLS is a common complication encountered during the initial presentation (as discussed above).

Hypokalemia

Hypokalemia can be due to inadequate intake or excessive losses. Excessive potassium losses commonly occur through diarrhea and vomiting, and also other sources such as gastrointestinal fistulae or cutaneous losses. Medications commonly used in children with cancer can also cause potassium losses through dysregulation of normal tubular handing handling and include amphotericin, cisplatin, ifosfamide, and glucocorticoids. While potassium derangements are typically limited to the time during medication use, significant numbers of pediatric patients treated with ifosfamide have demonstrated persistent hypokalemia long after completion of treatment. Uncommon causes of hypokalemia directly attributable to tumors include neuroendocrine neoplasms that can promote secretory diarrhea, ACTH-secreting tumors, and tumors that secrete either cortisol or aldosterone and through stimulation of mineralocorticoid receptors are able to induce aldosteronism and associated hypokalemia. Furthermore, acute myelogenous leukemia has been reported to result in direct renal tubular damage, with a putative mechanism attributed to lysozyme secretion from cancer cells [97]. Treatment for hypokalemia in these situations is typically with potassium supplementation.

Hypomagnesemia

There are several causes of hypomagnesemia in pediatric cancer patients, including poor oral intake, inadequate supplementation in parenteral nutrition, and excessive losses through diarrhea. Cisplatin is a common cause of drug-induced hypomagnesemia and can be improved with supplementation, but often appears to persist for years or longer after completion of treatment. Patients on calcineurin inhibitors typically need magnesium replacement as well. Rarely used in pediatric cancers, epidermal growth factor receptor inhibitors such as cetuximab and panitumumab prevent insertion of TRPM6 into the distal tubular apical lumen, which results in renal wasting of magnesium and can be diagnosed by measuring the fractional excretion of magnesium in urine. Unlike cisplatin, EGFR inhibitor-mediated hypomagnesemia improves following cessation of treatment.

Hypercalcemia

Hypercalcemia can occur by multiple different mechanisms in the cancer patients. One common cause is increased lysis of tumor cells either from high tumor burden or initiation of chemotherapy. Another mechanism is hypercalcemia of malignancy believed to be due to stimulation of osteoclasts via direct or indirect tumor cell activation. This is much less common in pediatric cancer than adult cancer; however, it can cause significant hypercalcemia. The spectrum of this presentation is quite broad, ranging from mild and asymptomatic to severe sequelae including coma and arrhythmia. Treatment focuses on enhancing urinary excretion of calcium and limiting the ongoing calcium liberation from bone. Aggressive hydration promotes calcium excretion from the kidneys, with the use of diuretics demonstrating additional benefit. Limiting osteoclast activity through the use of bisphosphonates has been employed with some benefit, although they have been demonstrated to cause renal tubular injury, FSGS, and reports of renal failure in pediatric patients [98]. The RANKL inhibitor denosumab has also been used as a treatment for significant hypercalcemia of malignancy [99]; however, there can be severe and refractory rebound hypercalcemia beyond discontinuation of denosumab, so extreme caution is warranted.

Hyperphosphatemia and Hypophosphatemia

Hyperphosphatemia is most commonly associated with TLS and exogenous administration of phosphate-containing fluids and medicine, especially in patients with reduced kidney function (due to AKI and/or CKD) also increases the risk for hyperphosphatemia. Appropriate volume expansion and limiting additional phosphate burden are the mainstays for management. Hypophosphatemia is seen in cancer patients undergoing chemotherapy and is typically due to a combination of poor oral intake due to cancer and associated treatments, as well as renal phosphate wasting from chemotherapeutic agents. Ifosfamide, cisplatin, and imatinib have all been demonstrated to cause phosphate wasting through Fanconi syndrome. Typical treatment is with phosphate supplementation, and this should be done with care in patients with decreased kidney function due to the risk of hyperphosphatemia.

Volume Status

Both hypovolemia and hypervolemia are significant concerns in cancer patients. Close monitoring of fluid intake and output is essential, as are accurate vital signs and daily weights, to monitor volume status. Anorexia, inadequate fluid intake, increased insensible and other fluid losses, diuretic use, sepsis, and third spacing are common causes for hypovolemia in cancer patients. These can occur simultaneously and place the kidney at risk for AKI and hypoperfusion. Conversely, fluid overload is common in the pediatric cancer population, and has been demonstrated to have severe negative outcomes, with modeling suggesting a 3% increase in mortality for each 1% of fluid overload, and patients with greater than 20% fluid overload are eight times more likely to die than patients with less than 20% fluid overload [87]. For pediatric oncology patients requiring acute dialysis treatment, fluid overload was a more sensitive predictor of need for RRT than creatinine or urine output [86, 100]. Therefore, careful evaluation of volume status, and prompt initiation of RRT, when indicated, may prevent excessive fluid overload and its associated increases in mortality.

Infection

Patients with cancer have a much greater risk of infection than that of the general population, with a relative risk of infection approaching 10 when compared to the general population [101]. There are multiple reasons for this increased risk, including tumor-induced immune dysfunction, chemotherapy and/or radiation-induced immune dysfunction, as well as disruption to innate immunity (including barrier function) due to medications, treatments, or invasive medical devices and instrumentation [102].

Fever in the neutropenic patient is a common inpatient and outpatient oncologic complication that can rapidly progress to critical illness if not promptly evaluated and treated. Underscoring the risk with fever in neutropenia, the mortality rate 50 years ago was greater than 90%, but with refinements in chemotherapy, more intensive evaluation and standardization of empiric treatment, that mortality rate has dropped to less than 1% [103], underscoring the necessity for a thorough work-up, as bacteria, fungi, and viruses can all cause significant illness. Typically the most common etiology for fever is bacteremia, but a prospective study showed that urinary tract infections can be as frequent a cause for fever in the neutropenic patient, underscoring the utility of urinalysis and careful evaluation for UTI [104]. In the cancer patient, the causative organisms for UTI do not differ significantly from those of the non-oncologic patient, and there is little data assessing the frequency of pyelonephritis relative to the general population – although there is additional concern for fungal pyelonephritis in the cancer population [102].

Given the concerns regarding significant immunosuppression and risk of complication with direct catheterization, the current recommendations recommend collection of a clean-catch or midstream (rather than direct catheterization) urine sample for culture (not relying on urinalysis for screening). Due to the risk of significant illness or death, urine collection should not delay antibiotic treatment [105]. Given the high rate of infection, prompt empiric treatment with antibiotics is utilized to prevent septic shock, which can have significant morbidity, including renal hypoperfusion and resultant AKI. Prophylaxis and treatment of such infections can include nephrotoxic antimicrobials, most notably vancomycin, aminoglycoside, amphotericin, foscarnet, acyclovir, and cidofovir. Careful monitoring of drug levels and collaboration with pharmacy is critical to optimize dosing, maximizing antimicrobial efficacy while limiting toxicity. When possible and based on the local epidemiology, alternative agents can be selected to decrease the risk of nephrotoxic AKI.

There are a wide variety of viral infections that cause morbidity in cancer patients, with several having a particular affinity of the kidney and genitourinary tract. BKPyV has minimal effect on the general immunocompetent population but has been demonstrated to cause reactivation and pathologic changes with immunosuppressive requirements from transplantation. The most common pathologic finding of BKPyV infection is hemorrhagic cystitis, with 18.7% of a HSCT cohort developing hemorrhagic cystitis, with 90% of them demonstrating evidence of BKPyV infection [106]. In addition, BKPyV can cause nephropathy, with renal biopsy evaluation demonstrating positive staining for virus, cytopathic changes in renal tubules, and interstitial nephritis [107].

BKPyV virus also infects the epithelial cells of the urinary tract and can cause ureteral and urethral stricture. BKPyV can be screened for by its presence in the urine, but this alone is not associated with development of nephropathy, so additional urine findings, including decoy cells and/or BKPyV aggregates known as “Haufen” (German for heap or pile), and evaluation of BKPyV viremia provide more diagnostic certainty [108]. Treatment of symptomatic BKPyV is typically reduction of immunosuppression (when possible) in a stepwise manner until remission is achieved, with antivirals such as cidofivir having some benefit, but effectiveness limited by their nephrotoxicity and other side effects. Hemorrhagic cystitis due to BKPyV is treated with hydration, bladder irrigation, pain control, and transfusions, as necessary [106].

Adenoviral infection is also associated with AKI and nephritis in the HSCT population, especially those with significant GVHD [109, 110]. EBV infection is implicated in a wide range of cancers, including Burkitt’s lymphoma and is largely responsible for the majority of post-transplant lymphoproliferative disorder seen following kidney transplantation, and is discussed in the Transplant Considerations section below.

Chronic Kidney Disease

Advances in oncology are leading to continual improvements in survival from the underlying cancer diagnosis. However, with increased survival, there is also increased risk for chronic sequelae following successful treatment, including a risk for CKD. Multiple studies have demonstrated a high risk for progression to CKD in critically ill cancer and HSCT patients [86, 111], with risk factors for progression to CKD including acute renal failure, total body irradiation, GVHD, long-term cyclosporine use, nephrectomy, cisplatin, carboplatin, ifosfamide, methotrexate, and abdominal radiotherapy [112, 46]. Data from the Childhood Cancer Survivor Study has shown an increase in the cumulative incidence of maintenance dialysis 15 years after enrollment from 0.5% in the 1970s to 0.9% in the 1990s [113], and a retrospective cohort study demonstrated a significantly elevated risk for end stage kidney disease (ESKD) for individuals with a history of childhood cancer when compared to the general population (hazard ratio 3.2; 95% confidence interval 1.3–7.7) [114]. The incidence of needing maintenance dialysis following HSCT is likely higher, estimated at 1–2% in several cohort studies of adult HSCT patients [57, 115, 116]. In one study, ESKD was 16 times more likely in patients following HSCT compared to the general population [116]. On the other side, the incidence of previous cancer among dialysis patients is understudied and analyses combining cancer and dialysis registries are needed. In a study of 74 French adults initiating dialysis with a previous diagnosis of cancer, survival at 1 and 2 years was similar compared to non-cancer dialysis patients matched for age and sex [117]. In a collaborative international study of >900,000 patients, approximately 5% of dialysis patients either had a previous diagnosis of cancer or primary renal disease caused by cancer or amyloidosis [118].

Growth

It is extremely common for patients with CKD, either pre-dialysis or on dialysis to demonstrate significant delay in linear growth, with the requirement for supplemental growth hormone. However, there have been concerns regarding the use of growth hormone in cancer patients and cancer survivors, based on reports of secondary neoplasms in patients using recombinant growth hormone. In a cohort of 172 pediatric cancer survivors, there was an increased incidence of secondary neoplasms [119]. While a rigorous study has yet to conclusively assess the relative risk of growth administration in cancer survivors, careful consideration and collaboration with the patient's oncologist, and potentially endocrinology, are warranted on case-by-case basis.

Nutrition

Nutritional considerations remain important in the management of patients with CKD to ensure adequate protein intake for growth and development. It is recommended that established expert guidelines are employed as a baseline for nutritional support, and adjusted as needed for the individual patient. Given anorexia, nausea, vomiting, and other limitations in achieving adequate enteral nutrition, consideration of tube feeds or parenteral nutrition may be necessary, as well as volume considerations in the oliguric or anuric patient.

Bone and Mineral Disease

Cancer and its associated treatments can cause impairment of peak bone mass, leading to lower bone mineral density, bone quality impairment, and avascular bone disease [120, 121]. With CKD progression to stages III–V, there is increased risk of renal osteodystrophy and associated derangements of phosphate and calcium handling, leading to hyperparathyroidism and significant bone disease. This is typically managed by vitamin D analogs, phosphate binders, and in more severe cases, calcimimetics and parathyroidectomy. Recently, the role of FGF23 and Klotho has been demonstrated in phosphate metabolism. Targeted FGF23 inhibition through medications such as burosumab is utilized in patients with X-linked hypophosphatemic rickets and was FDA-approved in 2020 for tumor-induced osteomalacia. No human studies have evaluated the use of burosumab in CKD patients, but animal studies demonstrated improvements in several parameters of hyperparathyroidism, but also significantly increased mortality [122], likely due to increased hyperphosphatemia. Therefore, use of burosumab in pediatric cancer patients should be very carefully considered, especially for those with demonstrated or likely reduced kidney function. Otherwise, given the lack of data in this population, following professional society guidelines for management of mineral-bone disease is warranted.

Anemia

Due to the kidney's role in generating erythropoietin, an essential hormone for the development of mature red blood cells, patients with advanced CKD and ESKD invariably develop significant anemia, which is effectively managed with the use of erythropoiesis-stimulating agents (ESAs) such as epogen and darbepoetin. Iron levels and stores are closely monitored, and supplemental iron is provided as needed. The use of ESAs in oncology patients requires careful consideration [123], especially given higher risk of thromboembolic events with ESA use in adults. Meta-analyses of ESA use in cancer patients demonstrated increased mortality both during active chemotherapy and beyond [124]. Proposed mechanisms of ESAs in promoting cancer have been improved oxygen delivery to tumor beds or off-target anti-apoptotic effects [123]. Despite laboratory evidence suggesting a supportive role in ESAs in tumorigenesis and tumor progression, a systemic review was not able to demonstrate that the use of ESA had an effect on tumor response [125].

Typically the recommendations for ESA use is limited in cancer patients, except when hemoglobin levels are below 10 g/dL [126] [127]. A few studies exist in the pediatric population and ESA use has demonstrated modest benefit in reducing transfusion dependence with chemotherapy-associated anemia, and is considered second-line to blood transfusions for managing chemotherapy-associated anemia in the pediatric population [128–130, 131]. There exist multiple recommendations for ESA usage in CKD patients who have completed their chemotherapy, but appear to suggest avoiding ESA treatment in patients with hemoglobin greater than 10 g/dL [132]. There is no clear data to guide when it is reasonable to use ESA in patients in remission.

Maintenance Dialysis

The choice of maintenance dialysis modality to treat ESKD in patients with a history of cancer requires multiple considerations. However, there is no absolute indication for initiation of a specific modality of dialysis. In a cohort of pediatric Wilms tumor patients with CKD requiring RRT, 56% started hemodialysis and 41% started peritoneal dialysis [133]. Many barriers for peritoneal dialysis initiation in adults, including age-related limitations and lack of family support [134], are often not as applicable in younger pediatric cancer survivors, but there remains a need for significant training and support from multiple caregivers in the household, which can sometimes be a limiting factor. Fastidious care of peritoneal catheters is essential, as bacterial peritonitis is a serious complication that can cause major complications, and fungal peritonitis requires catheter removal for definitive clearance of fungal infection. In addition to peritonitis concerns, careful technique must be used to avoid exit site and tunneled line infections. Also, peritoneal membrane failure due to previous abdominal surgery, intraabdominal infection, or irradiation may make peritoneal dialysis challenging or not possible, leaving hemodialysis, either in-center or at home, as the only reasonable option for long-term dialysis [135].

Regarding hemodialysis, achieving adequate blood flow rates requires stable central venous access, most commonly with a central venous catheter, which is often tunneled for greater stability and ease of care. For patients of appropriate size and adequate vascular anatomy, this tunneled line can be used while an arteriovenous (AV) fistula is generated and allowed to mature. In patients with active cancer, careful consideration of AV fistula should be considered, as there is no data in the pediatric population, and scant data among adult cancer patients that suggests a greater degree of early AV fistula failure, largely due to cancer mortality [136]. In all ESKD patients, but especially cancer patients, careful choice of location of vascular access should be considered, even from the onset of treatment, as patients with ESKD will have lifetime needs for central vascular access due to limited allograft survival and these sites should be preserved as much as possible. Brachial access, especially on the non-dominant arm, should be preserved for future AV fistula placement, and complications from iliac central line placement can complicate future allograft anastomoses and suitability for renal transplantation. Therefore, careful attention to peripheral and central venous access in patients with CKD after a diagnosis of cancer is imperative, often requiring close discussion with nephrology, surgery, and interventional radiology.

Kidney Transplantation

For patients with ESKD, dialysis is viewed as a bridge to transplantation, as pediatric kidney transplantation has superior outcomes for survival and quality of life [137]. In addition to the standard kidney transplant listing considerations, there are additional considerations in the oncologic patient, including diagnosis and staging of cancer, risk of recurrence, and duration of remission. Kidney transplant in survivors of childhood cancer is low, with one long-term study showing an incidence of 0.54% for receiving or being listed for a kidney transplant, but accounted for nearly half of all solid organ transplants in this population [138]. There are conflicting data regarding mortality in solid organ transplant patients with a prior history of malignancy in adult and inadequate studies to date in children, but a recent pediatric study demonstrated similar graft and patient survival rates for patients with or without prior malignancy [139].

Although the reported incidence of post-malignancy kidney transplant is low, both advances in cancer treatment regimens and the incorporation of new therapeutics with associated nephrotoxicities mean that the number of cancer survivors with ESKD requiring transplant will continue to increase over time. Apart from providing an impetus to minimize AKI during early cancer diagnosis and treatment, this increasing prevalence highlights the dilemma in determining optimal timing for transplantation. Traditionally, there has been concern about listing an ESKD patient shortly after cure or remission due to perceived risks of malignancy recurrence or short-term cancer mortality, with 2–5 years being the standard interval between remission and listing for an organ transplant, based on data demonstrated that more than half of cancer recurrences happened within 2 years of transplant, and 87% of recurrences within 5 years [140]. Despite this practice, there are several considerations that argue for a shorter time to kidney transplant [141]. The clear survival, morbidity, and QOL benefits should not be underestimated, especially in the developing pediatric patient. Also a comparison of long (>10 years) and short (0–2 years) intervals between cancer remission and kidney transplantation, there was no significant difference in all-cause mortality demonstrated, and this was observed in both all cancer and high-risk malignancies [142]. Furthermore, in a study of Wilms tumor patients, those with more aggressive disease requiring bilateral nephrectomy demonstrated markedly worse morbidity and mortality than more indolent Wilms tumor. However, those differences were ameliorated for those who received transplants early, with demonstration of excellent graft survival in patients with transplants performed as early 6 months following remission [133]. Intriguingly, a study demonstrated that infection-related and malignancy-related mortality was higher in dialysis patients than transplant patients, although this effect was blunted when excluding malignancy as the inciting cause for renal failure [143]. Therefore, a thoughtful evaluation of the risk of recurrence balanced against the benefits of transplantation is recommended in optimizing renal function with long-term survival.

Conclusion

Survival has dramatically improved for children treated for cancer or those undergoing a HSCT, but AKI and CKD remain common in this high-risk population, leading to morbidity and potential increases in non-relapse mortality. Close collaboration between nephrologists, oncologists, pharmacists, dieticians, endocrinologists, transplant providers, intensivists, and infectious disease experts is critical to maximize outcomes for these complicated patients. Careful attention to blood pressure, urine assessments for albuminuria/proteinuria, and kidney function with both serum creatinine and cystatin C is important in the long-term to screen for and manage CKD. As more patients are surviving longer, multi-disciplinary survivorship clinics are increasingly following those treated for cancer or having received a HSCT. Our understanding of the mechanisms of kidney injury has allowed the field of onconephrology to grow, and continued research in this area will hopefully further improve outcomes for patients in the future.

Cross-References

- ▶ Assessment of Kidney Function in Children, Adolescents, and Young Adults
- ▶ Chronic Hemodialysis in Children
- ▶ Drug Dosing in Abnormal Kidney Function in Children
- ▶ Evaluation and Management of Acute Kidney Injury in Children
- ▶ Nephrotoxins
- ▶ Pediatric Renal Tumors
- ▶ Progression of Chronic Kidney Disease and Nephroprotection in Children
- ▶ Renal Transplantation: Evaluation of Children and Donors

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Part IX

Hypertension

Pathophysiology and Epidemiology of Hypertension in Children

62

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Abstract

Hypertension (HTN) is a significant global health issue. It is the major risk factor for atherosclerosis, leading to the development of cardiovascular disease (CVD) as well as a risk factor for the progression to kidney failure. HTN has its antecedents during childhood, and atherosclerosis is already present in adolescents. This chapter overviews the many pathophysiologic alterations that can lead to the development of HTN. Primary and secondary HTN, the etiology of HTN along with normative values of blood pressure (BP), and the definition of HTN are also reviewed.

Keywords

Cardiovascular disease · Primary hypertension · Secondary hypertension · Target organ damage · Normative values · Ambulatory blood pressure · Pathophysiology · Epidemiology · Home blood pressure · White coat hypertension · Masked hypertension

Introduction

The goal of the first half is to provide the reader with an overview of the many pathophysiologic alterations that can lead to HTN. The latter half is devoted to address issues related to the epidemiology of HTN in children.

Mechanisms of Blood Pressure Regulation

BP is a function of cardiac output and systemic vascular resistance, and much has been learned about the many ways in which these two arms of the equation can be affected, down to the cellular level.

Cardiac Output and Systemic Vascular Resistance

HTN is fundamentally a hemodynamic disorder indicating a disturbance in cardiac output (CO) and/or systemic vascular resistance (SVR). CO is the product of stroke volume (SV), which is determined by preload, contractility, and afterload, and heart rate (HR), which is regulated by the sympathetic nervous system (SNS) and the cardiac autonomic pacemaker. SVR is the resistance to blood flow from systemic vasculature except for the pulmonary vasculature and is sometimes referred to as total peripheral resistance.

Although in adult patients primary HTN is usually associated with elevated SVR, pediatric primary HTN often results from increased HR and/or SV producing increased CO, as a consequence of excessive sympathetic activity. Li et al. reported that cardiac over-reactivity characterized by increased HR, left ventricular ejection velocity, and proximal pulse wave velocity may be the main cause of primary HTN in children [1]. Some of these findings may be gender specific, as the ENIGMA cohort showed that hypertensive males had elevated HR, SV, and CO, whereas hypertensive females had increased peripheral vascular resistance and pulse wave velocity (a measure of arterial stiffness) [2]. Patients with mild HTN have significantly higher CO, cardiac index (CI, a function of CO and body surface area), and sympathetic nerve burst frequency than age-matched controls. In borderline HTN, CI and HR are elevated and calculated SVR is normal, but in progressive HTN SVR increases and CO falls, indicating reduced vascular compliance. However, it is clear that not all hypertensive patients start out with increased CO. In some patients, HTN may develop because of increased SVR. In addition, some patients may develop HTN because of primary renal disease or a genetic defect that increases circulating blood volume through sodium retention.

Changes in Systemic Vascular Resistance and Vascular Walls

Previously, SVR was not thought to differ between normotensive and hypertensive adolescents and young adults. However, the recent Avon Longitudinal Study of Parents and Children (ALSPAC) showed that higher BP was associated with higher CO and higher SVR in a population of adolescents [3]. The ENIGMA cohort [4] showed that SVR was positively correlated with BP in overweight/obese young adults with hypertension, whereas CO was associated with SBP in lean young adults.

Because resistance to flow increases steeply with decreasing blood vessel diameter, SVR is mainly a function of small, peripheral arterioles, whose medial muscle layer thickens in response to sustained HTN. In experimental animals, after the onset of HTN, the earliest structural change observed in the arterioles is thickening of the media due to matrix deposition, smooth muscle cell hypertrophy (increase in cell size without division), and hyperplasia (increase in cell number). The medial smooth muscle cells then rearrange themselves to create a smaller lumen without change in cell number or size, a process termed remodeling. Finally, there may be resorption and loss of blood vessels in the periphery, or “rarefaction,” which has been described in both early and later stages of HTN. In larger vessels, the content of elastin and collagen in the media increases and the number of smooth muscle cells decreases (via medial atrophy, necrosis, or apoptosis), leading to a loss of elasticity and increased vessel “stiffness.” In summary, the hypertensive vessel wall is generally characterized by increased medial thickness or an increased media/lumen ratio of the resistance arterioles, resulting in greater resistance.

Altered vascular structure in HTN is accompanied by functional changes in the cellular components of the vessel wall, altering the balance of vasoconstrictive and vasodilatory mechanisms. Decreased relaxation has been attributed primarily to endothelial dysfunction leading to impaired production of vasodilatory substances including nitric oxide (NO) and prostacyclin. Increased contraction may also result from enhanced smooth muscle cell vasoreactivity, sensitivity to vasoconstrictors, and/or production of vasoconstricting substances such as endothelin and platelet-derived growth factor (PDGF). Smooth muscle cell responsiveness to vasoconstrictors can increase because of greater numbers of receptors or augmented contractile machinery. Moreover, vasoconstrictors frequently stimulate smooth muscle cell growth, and conversely, many growth factors have vasoconstrictor activity (e.g., PDGF is a vasoconstrictor, and angiotensin II is a potent smooth muscle cell growth factor).

Sympathetic Nervous System and Central Nervous System

The SNS plays a major role in BP regulation. Sympathetic output is physiologically regulated by baroreflexes and chemoreflexes to maintain BP, carbon dioxide, and oxygen tension. Sympathetic tone can be altered by a variety of factors including baroreflex dysfunction, obesity, salt intake, systemic inflammation, and psychological stress. Increased SNS activity leads to vasoconstriction and sodium retention. SNS activation is found in early stages of HTN and in uncontrolled severe HTN, and it promotes target organ damage such as left ventricular hypertrophy and increases cardiovascular risk. SNS activation is discussed in detail in the following section on primary HTN.

The renal sympathetic nerves innervate renal vessels, tubules, and the juxtaglomerular apparatus, and renal sympathetic nerve activity (RSNA) influences renal hemodynamics, solute and water handling, and hormone release. Increased RSNA is found in animal models of HTN and in hypertensive humans and acts to constrict the renal vasculature and decrease glomerular filtration rate (GFR) and renal blood flow. The hypertensive response to chronic renal adrenergic activation is associated with sustained increases in plasma renin activity and plasma angiotensin II (Ang II). Sympathetic nerve activation and circulating Ang II appear to have a cooperative effect, as experimental renal denervation in animals decreased the effects of Ang II on proximal tubule chloride and water reabsorption by 75%. A likely mechanism of this interaction is that, in addition to its direct effects on tubular cells, Ang II increases norepinephrine release from renal sympathetic nerve terminals. In experimental renal sympathetic nerve stimulation, Ang II enhanced the renal venous outflow of norepinephrine, an effect that was blocked by an Ang II receptor antagonist. These findings show that Ang II and RSNA work in concert to regulate solute and water handling by renal tubular epithelial cells.

A variety of interventional procedures targeted to the SNS have been tested for their effects on BP. Bilateral renal nerve ablation and carotid baroreceptor stimulation have been investigated as treatments for resistant HTN. Although renal denervation prevents or alleviates HTN in virtually all animal models, the renal denervation in patients with uncontrolled HTN (SYMPLICITY-HTN 3 trial) failed to demonstrate an effect on BP [5]. However, carotid baroreceptor stimulation reduced BP significantly in patients with resistant HTN.

Other potential antihypertensive treatments that modulate SNS function are renalase and catestatin. Renalase is a flavin adenine dinucleotide–dependent amine oxidase that is mainly secreted by the kidney and degrades circulating catecholamines (primarily epinephrine). Catestatin is a chromogranin A–derived peptide that blocks nicotine-stimulated catecholamine release. Both renalase and catestatin modulate cardiac function and systemic BP [6].

Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system (RAAS) (Fig. 1) is the major hormonal system regulating

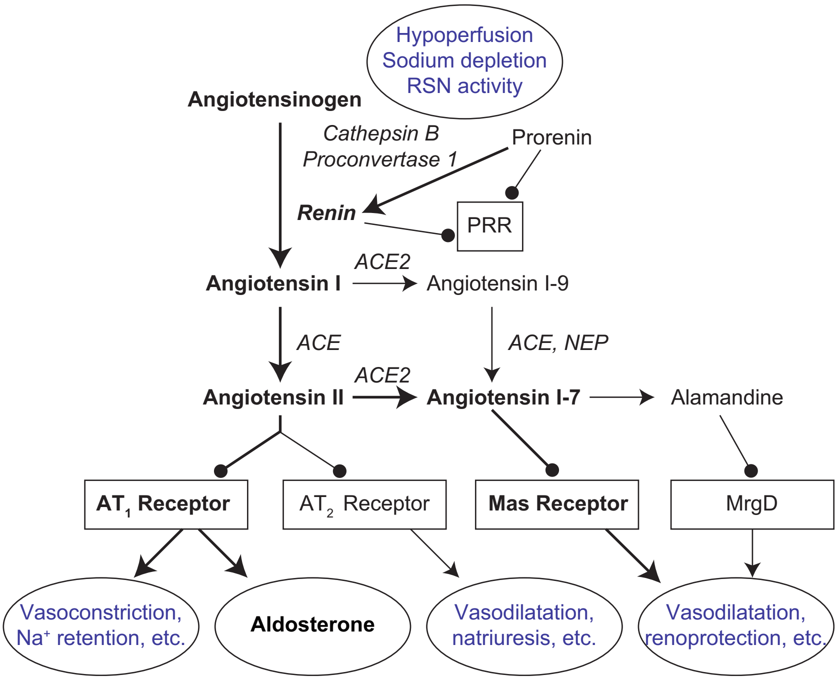
Hypoperfusion
Sodium depletion
RSN activity
Angiotensinogen
Cathepsin B
Proconvertase 1
Prorenin
Renin
PRR
ACE2
Angiotensin I
Angiotensin I-9
ACE
ACE, NEP
ACE2
Angiotensin II
Angiotensin I-7
Alamandine
AT1 Receptor
AT2 Receptor
Mas Receptor
MrgD
Vasoconstriction, Na+ retention, etc.
Aldosterone
Vasodilatation, natriuresis, etc.
Vasodilatation, renoprotection, etc.

Fig. 1 Renin-angiotensin-aldosterone system. PRR, pro-renin receptor; RSN, renal sympathetic nerve; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; AT_1 receptor, angiotensin II receptor type I; AT_2 receptor, angiotensin II receptor type II; MrgD, Mas-related G protein–coupled receptor D. Bold text indicates the major RAAS peptide hormones and their receptors

BP. The role of the RAAS in pediatric HTN and chronic kidney disease was recently reviewed [7]. Renin and its precursor prorenin are produced in the juxtaglomerular cells of the afferent renal arterioles and activate the RAAS. In addition, extrarenal sites of prorenin to renin conversion exist in many tissues. Major stimuli for renin secretion from the juxtaglomerular apparatus include glomerular underperfusion, reduced sodium intake, and SNS activity. In the classical systemic RAAS, circulating renal-derived renin cleaves hepatic-derived angiotensinogen to form angiotensin I (Ang I), which is converted by angiotensin-converting enzyme (ACE) in the lungs to the major active component of the RAAS, Ang II. ACE also degrades bradykinin, a potent vasodilator, into inactive metabolites. ACE exists in plasma, in the interstitium, and intracellularly. Tissue ACE is present in all major organs, including the heart, brain, blood vessels, adrenals, kidney, liver, and reproductive organs, where it augments the effects of circulating components of the RAAS.

The prorenin receptor, which is found on vascular smooth muscle cells, the distal tubule, and other locations [8], binds both prorenin and renin and facilitates the generation of Ang I from angiotensinogen. Free prorenin has no intrinsic enzymatic activity due to the presence of an N-terminal propeptide that obstructs access of angiotensinogen to the active site. However, prorenin bound to the prorenin receptor has enzymatic activity and, in addition, can be cleaved to form renin, increasing Ang I production. Rats overexpressing the prorenin receptor develop HTN, glomerular sclerosis, and proteinuria and have elevated plasma aldosterone concentrations, and polymorphisms of the prorenin receptor have been associated with human HTN.

Ang II is a potent vasoconstrictor of vascular smooth muscle and thus directly increases BP. Systemic vasoconstriction by Ang II reduces medullary flow, which activates sodium-hydrogen exchanger 3 (NHE3) and Na-K-ATPase in proximal tubules, leading to increased sodium reabsorption. Ang II also stimulates aldosterone release from the zona glomerulosa of the adrenal grand, which increases sodium reabsorption by activating the mineralocorticoid receptor and increasing expression of ENaC.

The effects of Ang II are primarily mediated by the type I and type II angiotensin receptors (AT_1R and AT_2R), both members of the G protein–coupled receptor family. AT_1R activation stimulates vasoconstriction and sodium retention, and excessive activation may lead to vascular cell hypertrophy and hyperplasia, reactive oxygen species generation, and inflammatory, thrombotic, and fibrotic processes [8]. These effects of AT_1R overactivation contribute to left ventricular remodeling, alterations in the morphology and mechanical properties of the vasculature, and endothelial dysfunction. Under physiologic conditions, the AT_2R mainly antagonizes AT_1R actions; its cardiovascular effects are generally opposite to those of the AT_1R and may be protective [9]. In the kidney, stimulation of the AT_2R promotes natriuresis through interactions with the renal dopaminergic system.

A membrane-attached homologue of the angiotensin-converting enzyme termed ACE2 hydrolyzes Ang I to angiotensin 1-9 [(Ang-(1-9)] and Ang II to angiotensin 1-7 [Ang-(1-7)] (Fig. 1) [10]. ACE2 is found in the heart, kidneys, testes, lungs, colon, small intestine, and ovaries. Both Ang-(1-9) and Ang-(1-7) are reported to oppose the functions of the classical RAAS. Reduced ACE2 levels are thought to contribute to primary HTN via impaired degradation of Ang II and reduced formation of vasodilator by-products in the renal endothelium [11]. ACE2 has recently received increased attention because it serves as a receptor of SARS-CoV-2 in nasal and oropharyngeal epithelia. Downregulation of ACE2 by SARS-CoV-2 may reduce Ang-(1-7) production while increasing Ang II levels and AT_1R activation, which may contribute to acute lung injury [12].

Ang-(1-7), the major product of ACE2, is present in the circulation and in many tissues, including heart, blood vessels, and kidney. Ang-(1-7) acts via the G protein–coupled receptor Mas (MasR) [13], which is found mainly in the brain, heart, kidneys, and testicles. Ang-(1-7) regulates renal function by acting at glomerular and tubular sites. While Ang II markedly raises efferent glomerular arteriolar resistance without changing afferent arteriolar resistance, Ang-(1-7) vasodilates afferent arterioles and increases renal blood flow [14]. In vitro studies and findings in anesthetized animals have suggested that Ang-(1-7) acts as a natriuretic/diuretic hormone. In humans, there is increasing evidence that Ang-(1-7) may be involved in the development of primary HTN and the progression of chronic kidney disease [7]. The ACE2/Ang-(1-7)/MasR axis may be a target for fetal programming of cardiovascular and renal function, and a deficiency of Ang-(1-7) may contribute to the increased risk of hypertension and chronic kidney disease in premature infants [15]. Another vasodilator peptide, alamandine, is produced by decarboxylation of the N-terminal aspartic acid of Ang-(1-7) to alanine. Alamandine causes endothelium-dependent vasodilation via the Mas-related G protein–coupled receptor (MrgD). It is found in human plasma and is elevated in ESKD patients [16].

Aldosterone

Aldosterone is produced from deoxycorticosterone by the mitochondrial enzyme CYP11B2 (aldosterone synthase) [17]. As discussed later, mutations in the CYP11B2 gene lead to glucocorticoid-remediable aldosteronism, a monogenic cause of HTN. Aldosterone synthase is expressed in the zona glomerulosa of the adrenal glands, aorta, endothelial cells, vascular smooth muscle cells, hypothalamus, hippocampus, amygdala, and cerebellum. Ang II and Ang III induce aldosterone synthesis, and potassium, endothelin, adrenocorticotropic hormone (ACTH), and vasopressin stimulate aldosterone secretion. Inhibitors of aldosterone secretion include atrial natriuretic peptide, somatostatin, and dopamine. Dietary sodium restriction increases aldosterone secretion, which acts to restore plasma volume. Aldosterone binds to intracellular, high-affinity type I mineralocorticoid receptors that are present in the kidney, brain, heart, colon, and vessel walls. Aldosterone receptor activation induces sodium retention and potassium excretion and enhances sympathetic nervous system function by decreasing norepinephrine uptake in the myocardium. Aldosterone signaling can also cause baroreceptor dysfunction [18] and eventually myocardial and vascular fibrosis [19]. The importance of aldosterone in HTN and cardiovascular disease is highlighted by studies demonstrating that the aldosterone receptor antagonists spironolactone and eplerenone improve endothelial dysfunction and resistance artery stiffness, increase NO bioactivity in chronic heart failure and CKD, reduce cardiac fibrosis, and prevent the development of nephrosclerosis and cerebrovascular lesions [17].

Dopamine

Dopamine is an important modulator of systemic BP with direct actions on the heart, arteries, veins, and kidney. Dopamine is synthesized in sympathetic nerve terminals, and also in non-neural tissues (e.g., kidney and gastrointestinal tract). Dopamine modulates renal epithelial sodium transport and gastrointestinal sodium uptake [20], and it can indirectly modulate BP via release of hormones and humoral agents such as aldosterone, catecholamines, endothelin, prolactin, pro-opiomelanocortin, renin, and vasopressin [21]. Dopamine also modulates fluid and sodium intake via appetite centers in the brain and regulates BP by direct action on neuronal cardiovascular control centers. Dopaminergic blockade and decreased function of dopaminergic receptors are both associated with the development of HTN in rats [20]. Five dopamine receptor subtypes have been identified, which can be divided into D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) receptors [20]. Stimulation of D_1 and D_5 dopamine receptors, which are expressed in vascular smooth muscle cells, can cause vasorelaxation, whereas stimulation of D_3 receptors produces either vasodilatation or vasoconstriction, depending on the degree of renal nerve activity. Disruption of the D_3 receptor gene in mice is associated with renin-dependent HTN and a decreased ability to excrete an acute saline load [22]. Fenoldopam, an intravenously administered selective D_1 receptor partial agonist, lowers BP by stimulating peripheral D_1 receptors. Dopamine also activates myocardial β-adrenergic receptors, increasing CO, and plays an important role in modulating vascular smooth muscle tone. At extremely high concentrations, dopamine occupies α_1-adrenergic receptors, producing vasoconstriction.

Dopamine also regulates the expression of other receptors involved in BP regulation (e.g., endothelin-B receptor and AT_1R). Both D_1- and D_2-like receptors downregulate AT_1R expression and function. Aberrant dopaminergic regulation of aldosterone secretion may be involved in the development of some forms of hyperaldosteronism and HTN [21].

Natriuretic Peptides

Natriuretic peptides (NPs) are vasoactive peptides that modulate natriuresis and BP, and also have many other biological activities. At least eight NPs have been described, including atrial natriuretic peptide (ANP), B-type (BNP), C-type (CNP), *Dendroaspis* natriuretic peptide (DNP), urodilatin, uroguanylin, osteocrin, and musclin. Three natriuretic peptide receptors (NPR-A, NPR-B, and NPR-C) have been identified and function as transmembrane guanylyl cyclases, and the related protease convertases (furin, corin, and PCSK6) are known to contribute to NP systems [23].

ANP and BNP have similar biological functions, including potent diuretic, natriuretic, and vasorelaxant effects. In the kidney, they increase GFR by vasodilatation of the afferent arteriole and vasoconstriction of the efferent arteriole, and they promote natriuresis by inhibiting tubular sodium reabsorption and renin and aldosterone biosynthesis. ANP and BNP may reduce sympathetic peripheral vascular tone by blocking vasoconstrictive effects of the RAAS and the SNS. Administration of ANP reduces BP in humans and in animal HTN models by reducing systemic vascular resistance. NPs are cleaved by the zinc-dependent metalloprotease neprilysin, leading to catalytic degradation. The effects of neprilysin on NPs may play an important role in BP control and cardiac function [24].

Endothelin-1

Endothelin-1 (ET-1) is a potent endothelial-derived vasoconstrictive peptide [25]. Three isoforms of endothelin (ET-1, ET-2, ET-3) encoded by separate genes have been identified. ET-1, the most abundant isoform, primarily acts in a paracrine fashion, signaling through ET_A and ET_B receptors. Via the ET_A receptor, ET-1 closes membrane K+ channels, leading to membrane depolarization and smooth muscle cell contraction. In general, activation of the ET_A receptor causes vasoconstriction, whereas the ET_B receptor causes vasodilation and induces production of NO and prostacyclin. The ET_A receptor can also stimulate cell proliferation, while the ET_B receptor prevents apoptosis. In the kidney, ET-1 constricts afferent and efferent glomerular arterioles, reducing renal blood flow and glomerular filtration rate. It also blocks sodium reabsorption by inhibiting tubular Na^+-K^+- ATPase activity in the proximal tubule and collecting duct. ET_B knockout mice have HTN secondary to renal sodium retention, and there is evidence of enhanced vascular ET-1 expression in salt-sensitive hypertensive rat models [26].

Patients with HTN are found to have normal circulating ET-1 levels; however, they have elevated local vascular or renal ET-1 levels. Many ET receptor antagonists have been studied and are found to reduce BP effectively. A recent clinical trial of the ET receptor antagonist aprocitentan in patients with primary HTN showed significant BP reduction [27]. However, although the drug was well tolerated, increased plasma volume and weight gain were noted in patients with a high-sodium diet [28]. Currently, the efficacy of ET receptor antagonists for resistant and refractory HTN is under investigation.

Other Vasoactive Molecules

In addition to the factors described above, many other vasoactive molecules can regulate BP. Because of limited space, the functions of several other vasoactive molecules are summarized in Table 1.

Circadian Rhythm

The circadian rhythm is an intrinsic 24-h cycle that affects numerous physiologic processes. Vascular tone, SVR, heart rate, and BP increase in the early morning hours in both normotensive and hypertensive subjects. These changes correspond to increased plasma renin activity and a surge in catecholamine secretion. BP peaks at approximately 9 a.m. and is lowest at 3 a.m. A significant rise in BP occurs prior to awakening. Diurnal variation of BP has been clearly demonstrated in normotensive children; in hypertensive children, data suggest that diurnal variation is preserved in primary HTN but is abnormal in secondary HTN [29]. Hypertensive patients with blunted BP

Table 1 Other vasoactive agents and their function

Vasoactive molecules	Function
Bradykinin	Vasodilation Increased PGE_2, NO, and natriuresis via bradykinin 2 receptor
Nitric oxide	Endothelium-derived relaxing factor
Prostaglandins	Vasodilation Inhibition of platelet aggregation
Vasopressin	Increased water absorption Arterial vasoconstriction
Urotensin II	Potent vasoconstriction
Adenosine	Vasodilation via A_{2A} and A_{2B} receptors TGF via A_1R, vasoconstriction of afferent arterioles, reduced GFR Vasodilatation and increased medullary blood flow via A_2R
Adrenomedullin	Vasodilation, natriuresis, diuresis, and inhibition of aldosterone production

PGE_2 prostaglandin E_2, *NO* nitric oxide, *TGF* tubuloglomerular feedback, *GFR* glomerular filtration rate

dipping have increased risk of cardiovascular target organ damage and cardiovascular morbidity and mortality [30].

The circadian pacemaker in mammals is located in the suprachiasmatic nucleus (SCN) in the anterior hypothalamus. It receives input about light/dark cycles from the retina via the retinohypothalamic tract, and produces several neuropeptides, including vasopressin and vasoactive intestinal peptide (VIP), which appear to have diurnal peaks of expression and release [31]. Animal experiments have demonstrated that the SCN is linked to multiple sympathetic pathways that affect many components of the cardiovascular system, and it plays a crucial role in synchronizing the peripheral oscillators that are found in all tissues. The primary mediator of SCN action appears to be melatonin, which is secreted by the pineal gland. Melatonin appears to transmit information about light/dark cycles from the SCN to the peripheral oscillators and to other centers in the hypothalamus that regulate cardiovascular activity [32].

Supporting evidence for the role of the SNC and melatonin in BP regulation comes from studies demonstrating that melatonin levels are decreased in hypertensive individuals, and that administration of melatonin lowers BP. Autopsy studies of patients with primary HTN have demonstrated that SCN neuronal populations that produce vasopressin and VIP are reduced, suggesting that in at least some individuals with HTN, normal function of the intrinsic biologic clock is impaired [33]. Melatonin appears to modulate activity of the SNS, has antioxidant activity, and may prevent endothelial dysfunction [34].

The Gut Microbiome

The gut microbiome is recently recognized to be an important neuroimmunological modulator. A systematic review and meta-analysis of the effect of probiotics on BP showed that consumption of probiotics significantly reduced both systolic and diastolic BP [35]. Increased dietary sodium intake may lead to gut dysbiosis, inflammatory cytokine release, and elevated BP. Changes in the gut microbiome may also increase BP via G protein–coupled receptors such as Gpr41, Gper1, and olfactory receptors [36].

Renal Mechanisms

The kidneys respond to changes in arterial pressure by altering the excretion of salt and water; increases in renal perfusion pressure lead to decreases in sodium reabsorption and increases in sodium excretion (pressure natriuresis). The mechanism of pressure natriuresis is related to medullary blood flow, renal interstitial hydrostatic pressure (RIHP), and renal autocoids (NO, prostaglandins, kinins, and Ang II). Increased renal perfusion pressure leads to increases in RIHP, NO, prostaglandin E2, and kinins, and a decrease in Ang II. Increased blood flow to the renal medulla, which normally receives only 5–10% of the total renal blood flow, contributes to washout of the urea gradient. This results in an increase in RIHP, which in turn reduces tubular reabsorption of sodium and water, leading to natriuresis and diuresis, and thereby lowering BP.

Renal autoregulation, or the kidney's intrinsic ability to maintain constant renal blood flow and GFR despite changes in renal perfusion pressure, serves to protect nephron function. However, major variations in renal perfusion can still influence renal excretory function, renin release, and arterial BP. Autoregulation occurs in interlobular arteries, side branches of arcuate arteries, and afferent and efferent arterioles. It depends on myogenic mechanisms present in those vessels, among the most important of which is calcium entry through voltage-gated calcium channels. Tubulo-glomerular feedback also plays an important role in autoregulation. Substantial evidence exists that the primary role of these renal autoregulatory mechanisms is to protect the kidney from damage caused by fluctuations in BP.

Renal autoregulation is reset in animal models of spontaneous HTN. Ang II plays an important role in resetting the autoregulation limits. In the spontaneously hypertensive rat, higher pressures are needed to trigger the vasoconstrictive response in the afferent arteriole, but in the

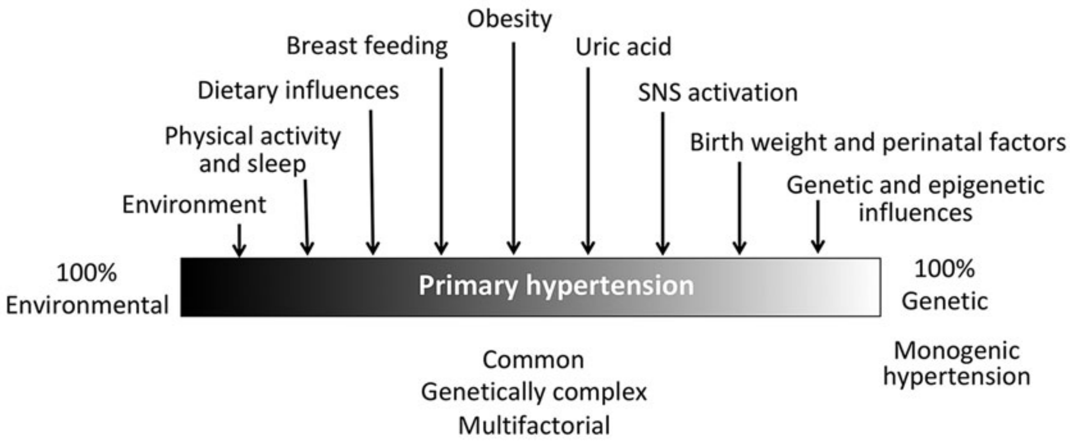
Obesity
Breast feeding
Uric acid
Dietary influences
SNS activation
Physical activity and sleep
Birth weight and perinatal factors
Environment
Genetic and epigenetic influences
100% Environmental
Primary hypertension
100% Genetic
Common
Genetically complex
Multifactorial
Monogenic hypertension

Fig. 2 Factors contributing the development of primary hypertension. SNS, sympathetic nervous system

intermediate portion of the interlobular artery a lower threshold pressure stimulates a greater myogenic response [37]. In the Dahl salt-sensitive hypertensive rat, both the afferent arterioles and the interlobular arteries have reduced myogenic responsiveness to increases in perfusion pressure [38]. Abnormalities in renal autoregulation are more pronounced in patients with severe HTN than in those with moderate HTN [39], suggesting that chronic HTN can induce alterations in renal autoregulation. Thus, disturbed renal autoregulation may contribute to the perpetuation of BP elevation in patients with established HTN.

Finally, the kidney also controls salt and water balance by regulating the number and function of critical sodium transporters and channels. For example, in an animal model of primary HTN, the Dahl salt-sensitive hypertensive rat, Ang II/AVP (arginine vasopressin) receptor dysfunction produces HTN through increased tubular sodium and fluid reabsorption [40]. Expression of many of these proteins is influenced by aldosterone and other hormones that affect BP. As discussed later in this chapter, all monogenic forms of human HTN described thus far can be characterized by alterations in renal ion channels leading to either increased sodium reabsorption or decreased excretion. Thus, the kidney plays a central, complex role in the pathophysiology of HTN.

Primary Hypertension

Primary HTN is a multifactorial disorder in which elevated BP is caused by different combinations of contributing factors described above in different individuals. The following sections will highlight some of the more important mechanisms likely to contribute to the development of primary HTN in children (Fig. 2).

Genetic and Epigenetic Influences

The reason for the cluster of primary HTN within families is partly due to genetic susceptibility. The rare alleles responsible for the monogenic disorders do not associate with primary HTN. Primary HTN is thus considered to be the product of multiple genetic variants interacting with each other. Genome-wide linkage studies using families or siblings reported significant or suggestive linkage for HTN, the results of which were variable. Many association studies (case-control studies) with candidate genes for HTN have been reported, most of which failed to replicate. Genome-wide association studies (GWAS) is an approach that involves rapidly scanning markers across the complete sets of DNA or genomes, of patients and controls to find genetic variations associated with the disease. More than 50 GWAS identified a total of more than 30 common loci associated with primary HTN [41]. While a few novel pathways have been identified which may be related to the pathogenesis of HTN, the clinical utility of GWAS data is at present limited.

Epigenetics is a mechanism that regulates gene expression independently of the sequence of DNA. Evidences are accumulating that epigenetics play an important role in the development of HTN capturing environmental and lifestyle factors [42]. DNA methylation and histone modifications are the two major epigenetic mechanisms. Elevated 11-ß-hydroxysteroid dehydrogenase (11ß-HSD2) promoter methylation was shown to be associated with primary hypertensives with lower activity of 11ß-HSD2. The modulation of the promoter region of WNK4, a regulator of sodium reabsorption, by histone acetylation is another example of epigenetic involvement in the development of salt-sensitive HTN [42]. Agnostic epigenome-wide association studies (EVAS) became also possible, but few related to HTN have been reported [41]. More recently, studies of noncoding RNA that regulate gene expression post-transcriptionally in HTN have been reported [43]. Transcriptomic approach examining RNA expression profile is also being tried. Epigenetic modifications reported in HTN thus far has been summarized recently [44].

Influence of Birth Weight and Perinatal Factors

The findings of Barker et al. relating low birth weight (LBW) to increased risk of death from cardiovascular disease (CVD) in later adult life have led to “developmental origins of health and disease (DOHaD)” concept. An inverse association between BP and birth weight supports the concept although the magnitude is small (−1.5 to −2.0 mmHg/kg). The association was independent of potential confounding factors such as socioeconomic status, maternal and participant smoking, maternal age, and others. Accelerated postnatal growth especially weight gain in LBW individuals was associated with increased BP. A meta-analysis concluded that the inverse linear association primarily exists between birth weight and SBP. Of note, a recent GWAS of birth weight identified 190 loci explaining variance in birth weight and higher adult BP suggesting that the inverse birth weight–BP association is genetic rather than developmental [45].

Most of the initial studies did not make a distinction between LBW as a result of intrauterine growth restriction and prematurity. The role of prematurity per se for the development of HTN was supported by a systematic review and a meta-analysis, which concluded that infants born preterm have modestly higher SBP later in life and may be at increased risk for developing HTN and its sequelae [46]. The effect of prematurity, however, could not be differentiated from that of LBW due to prematurity although not due to intrauterine growth retardation.

Brenner et al. proposed that LBW might be associated with a reduced nephron endowment leading to reduced renal sodium excretion and increased susceptibility to HTN. This hypothesis was supported by studies that showed a relation between birth weight and nephron number. Also, hypertensive white patients have been shown to have significantly fewer glomeruli per kidney than matched normotensives although birth weight was not reported in the study.

Vascular factors (microcirculatory rarefaction, endothelial dysfunction, and arterial stiffness) and hypothalamus-pituitary-adrenal axis are also considered to play a role in the pathogenesis of developmental origins of primary HTN.

Animal studies have supported the epidemiologic evidence. Rat dams fed low-protein diet bear young that are small at birth and later develop HTN. A decreased nephron number was observed in the offspring. Many other DOHaD models have supported various mechanisms of developmental origins of HTN observed in humans.

Other than LBW, maternal smoking, alcohol consumption, obesity, diabetes, and preeclampsia during pregnancy as well as bottle-feeding of newborns have been reported to lead to HTN later in life.

Sympathetic Nervous System Activation

Many evidences suggest that SNS activation/overactivity is the primary mechanism by which BP elevation is initiated in young individuals with primary HTN. Children with primary HTN have resting tachycardia compared to normotensives, and resting heart rate has been found to correlate with BP in epidemiologic studies.

Another manifestation of SNS activation in hypertensive children is heightened cardiovascular reactivity to stress. Hypertensive adolescents had significantly greater increases in heart rate, SBP, and DBP during mental stress than normotensives. Increased cardiovascular reactivity to the cold pressor test predicts the subsequent development of HTN. An interaction between genetic factors and SNS activation is suggested by differences in the cardiovascular response to stress in children with different racial backgrounds or family histories of HTN. Recent evidences suggest a role of several neuropeptides such as neuropeptide Y (NPY), a strong vasoconstrictor, mediating the effects of stresses and sympathetic nerves on BP. A common genetic variation at the human *NPY* locus increases its transcription, systemic vascular resistance, and heritable responses to environmental stress, resulting in elevation of BP [47].

SNS activation in primary HTN is supported by several other evidences. Both children and young adults with primary HTN have elevated plasma norepinephrine levels. Altered heart rate and BP variability as well as hyperkinetic circulation (increased ejection volume) have also been associated with primary HTN in children.

Increased SNS activity has been found in microelectrode recordings from both peripheral and renal sympathetic nerves in hypertensive individuals. There is also substantial evidence to suggest that SNS overactivity may be involved in the development of the vascular hypertrophy, left ventricular dysfunction, and other systemic cardiovascular effects of established HTN. The importance of cardiac component and increased aortic stiffness rather than increased systemic vascular resistance has recently been suggested [1].

Obesity

The prevalence of HTN in obese children is higher. The childhood obesity epidemic appears to be associated with an increase in prevalence of HTN in children. Total body fat mass positively correlates with increased BP. The correlation was stronger for SBP than DBP. In a similar manner to adults, central body fat is more strongly related to BP than peripheral fat in children.

Insulin resistance plays a role in the development of high BP by increasing renal sodium reabsorption (Fig. 3). Increased renal sympathetic nervous activity seems to be responsible for this effect. The second mechanism is through effects on vascular structure and function. While insulin acts as a vasodilator, in hypertensive subjects, this effect is offset by vasoconstriction mediated by increased sympathetic nervous activity. Insulin may also act to stimulate vascular smooth muscle proliferation, leading to vascular medial hypertrophy and increased peripheral vascular resistance.

Leptin, a peptide produced by adipose tissue, is strongly associated with BP. With weight gain, leptin increases to counteract obesity. In obese hypertensives, leptin levels are elevated and correlate with BP [48]. The major link between hyperleptinemia and HTN appears to be increased SNS activity, as demonstrated in animal studies. Leptin also has significant intraglomerular effects, resulting in glomerular sclerosis by increasing TGF-β synthesis. In contrast to leptin, adiponectin inversely correlated with BP in obese adolescents. Unlike other adipocitokines including leptin, adiponectin expression and serum concentration are reduced in obesity.

Activation of the RAAS, oxidative stress, and inflammation are also involved in the mechanisms of obesity HTN, although theses mechanisms and insulin resistance are operative also in nonobese hypertensives. Children with obesity and primary HTN have been demonstrated to have elevated plasma renin activity. In a study conducted in adolescents, urinary levels of F2-isoprostane, a marker of oxidative stress, correlated with body fat and with diastolic BP. Furthermore, immune abnormalities and inflammation have been reported in children with obesity [49].

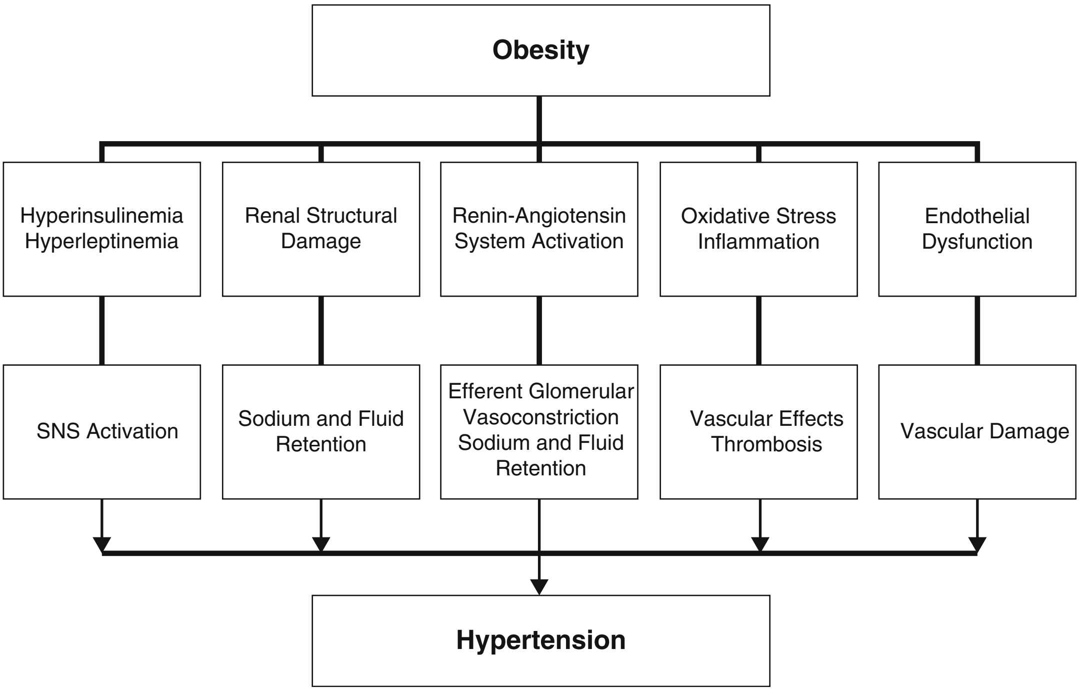
Obesity
Hyperinsulinemia Hyperleptinemia
Renal Structural Damage
Renin-Angiotensin System Activation
Oxidative Stress Inflammation
Endothelial Dysfunction
SNS Activation
Sodium and Fluid Retention
Efferent Glomerular Vasoconstriction Sodium and Fluid Retention
Vascular Effects Thrombosis
Vascular Damage
Hypertension

Fig. 3 Mechanism of hypertension in obesity. SNS, sympathetic nervous system. The figure is created by IY and Dr. Joseph Flynn

Finally, obesity produces direct effects on the kidney that contribute to the development of HTN. The kidneys are compressed by the perirenal fat, which leads to increased interstitial pressure, reduced medullary blood flow, and tubular compression, resulting in increased sodium reabsorption [48].

Dietary Influences

While BP is positively associated with sodium intake in adults, evidence for a direct relation between sodium and BP has been less conclusive in children. A recent systematic review and meta-analysis of experimental and observational studies in children and adolescents concluded that sodium intake is positively correlated with BP [50]. SBP and DBP increase approximately 1 mmHg for the addition of 1 g per day of sodium.

Dietary sodium intake early in life may affect BP in later life. Geleijnse et al. compared infants receiving low or normal sodium-containing infant formula for the first 6 months of life. A significant decrease in BP was found at 6 months and 15 years of age in children fed the lower sodium formula [51].

Potassium supplementation is associated with a decrease in BP in adults. Studies in children, however, gave conflicting results. Longer-term supplementation trials with potassium in hypertensive children induced lower age-related BP increases in SBP and DBP compared with non-supplemented children. A systematic review and meta-analysis found that increasing potassium intake decreased SBP and DBP by 0.28 mmHg and 0.92 mmHg, respectively, in children [52]. In the above-mentioned recent systematic review and meta-analysis, the association between sodium and BP is stronger among children with a low potassium intake suggesting a role to enhance urinary sodium excretion [50].

Uric Acid

Uric acid levels were suggested to be indicators of adolescents at risk for HTN. Subsequently, uric acid was proposed to play a role in primary HTN by contributing to endothelial dysfunction leading to microvascular and inflammatory injury to the kidney. In a short-term prospective study, treatment with allopurinol resulted in reduction of BP in adolescents with newly diagnosed HTN. A follow-up randomized, double-blinded, placebo-controlled trial comparing two mechanisms of uric acid reduction, allopurinol or probenecid, in prehypertensive obese adolescents concluded that uric acid causes increased BP that can be mitigated by uric acid–lowering therapy.

Animal experiment studies suggest that an increase in intracellular uric acid is important for the pathophysiology of primary HTN [53]. Cellular actions of uric acid include inflammation, oxidative stress, immune activation, and release of vasoactive agents. While GWAS did not support the casual association of serum uric acid and HTN, this was considered to be because cellular uric acid is more important for the pathogenesis. Raised BP has been shown to be related to sugar, fructose, and salt in the diet through various mechanisms including uric acid production [54].

Physical Activity and Sleep

There was an inverse dose–response association between levels of recreational physical activity and risk of HTN in adults. In children and adolescents, however, a meta-analysis concluded that short-term exercise does not appear to reduce resting BP. Recent randomized controlled trials assessed the influence of combined lifestyle changes including both diet and exercise on multiple cardiovascular risk factors in overweight adolescents. Although it is difficult to interpret the influence of exercise alone on BP, most studies suggest that physical activity can effectively reduce BP in children. A recent meta-analysis reported that school-based physical activity interventions improved DBP [55]. Physical activity has been shown to be associated with improved vascular structure and function, which is likely to contribute reduction in BP. Sedentary activities are suggested to be more than the opposite of physical activities being associated with SBP after adjustment for confounding factors.

Sleep disorders including sleep apnea are associated with HTN and cardiovascular morbidity in adults. Findings from pediatric studies also suggest that elevated BP is associated with sleep disorders. Several cross-sectional studies have described trends of increasing BP with greater frequency of apnea and hypopnea during sleep in children. In a random sample of the local elementary school children, sleep-disordered breathing was significantly associated with higher levels of SBP. A recent systematic review found an association with sleep (duration, timing, or quality) and HTN in children and adolescents [56].

Breastfeeding

Breastfeeding has been found to be associated with reduced BP in later life, although the findings have not been consistent. Martin et al. conducted a meta-analysis of studies published after 1980 reporting on BP levels in breast- and bottle-fed subjects. Pooled estimates based on a random effects model indicated that breastfeeding was associated with a 1.4 and 0.5 mm Hg reduction in SBP and DBP, respectively. The magnitude of the association is similar to those reported for salt restriction and physical activity. According to a recent systematic review, there is insufficient evidence to draw conclusions about CVD outcomes and infant feeding [57]. There were some evidences that feeding less or no breast milk is not associated with childhood HTN.

The putative association of breastfeeding and lower BP may be explained by a hormonal effect, lower sodium intake, or a protective effect of long-chain polyunsaturated fatty acids that are not present in most formula.

Environment

Adverse childhood socioeconomic status is associated with increased CVD in later life independently of adult socioeconomic status. Children from the low socioeconomic status are reported to have higher BP in adulthood than those from high socioeconomic status although other studies do not support the association. A systematic review and a meta-analysis suggested an inverse association of childhood adverse socioeconomic status and adulthood inflammation, which may possibly explain the association between the former and HTN [58]. Air and noise pollution as well as acute and chronic stress have also been associated with primary HTN in children.

Secondary Hypertension

Major causes of secondary HTN are summerized in Table 2.

Table 2 Differential diagnosis of childhood hypertension

System	Condition
Primary hypertension	
Metabolic syndrome	Dyslipidemia, impaired glucose tolerance, central obesity
Renal	Renovascular disease (syndromic or nonsyndromic) Fibromuscular dysplasia Mid-aortic syndrome Renal artery aneurysm Atherosclerotic renal artery stenosis Transplant renal artery stenosis
	Renal artery or vein occlusion or compression
	CAKUT
	Cystic kidney disease
	Glomerulonephritis Renal infarction
	Monogenic disorders of salt and water handling
Cardiac	Coarctation of the aorta
	Aortic stenosis
Vasculitis	Polyarteritis nodosa
	Takayasu arteritis
	Systemic lupus erythematosus ANCA disease
Endocrine	Thyrotoxicosis
	Cushing syndrome
	Acromegaly
	Hypercalcemia
	Congenital adrenal hyperplasia
	Pheochromocytoma and paraganglioma
	Renin secreting tumor
Neurologic	Intracranial mass
	Intracranial hemorrhage
	Autonomic dysfunction
	Pain
	Anxiety
	Seizures
Respiratory	Chronic lung disease
	Obstructive sleep apnea
Pregnancy-associated	Hypertension exacerbated in pregnancy (Geller syndrome) Eclampsia/Pre-eclampsia Gestational hypertension
Monogenic disorders	Apparent mineralocorticoid excess Congenital adrenal hyperplasia Primary glucocorticoid resistance Liddle's syndrome Pseudohypoaldosteronism type II

(continued)

Mendelian Forms of Hypertension

Molecular genetic studies have identified many gene mutations that affect BP. Below, we will discuss well-characterized monogenic hypertensive disorder [59]. Many of these disorders lead to aberrant distal tubular sodium reabsorption and volume expansion due to hyperaldosteronism, defects in steroid biosynthetic enzymes, and activation of sodium channels or transporters (Fig. 4) [60].

Glucocorticoid-remediable aldosteronism (GRA) or familial hyperaldosteronism type I (FH-I) is an autosomal dominant disease characterized by early onset of HTN with normal or elevated aldosterone levels despite suppressed plasma renin activity. GRA is caused by a chimeric gene that results from unequal crossing-over between the aldosterone synthase (*CYP11B2)* gene and the 11β-hydroxylase gene (CYP11B1) on chromosome 8. Aldosterone synthase is the rate-limiting enzyme for aldosterone biosynthesis in the adrenal glomerulosa, and 11β-hydroxylase is involved in cortisol biosynthesis in the adrenal fasciculata whose expression is regulated by adrenocorticotropic hormone (ACTH). The resulting chimeric gene (*CYP11B1/CYP11B2*) is expressed in the adrenal fasciculata and encodes a protein with aldosterone synthase enzymatic activity whose expression is regulated by ACTH.

Table 2 (continued)

System	Condition
	Hypertension with brachydactyly Mutations in PPAR-gamma
Iatrogenic	Medications
	Salt and water overload
	Umbilical artery catheterization
Illicit drug use	Cocaine, others
Environmental toxins	Cadmium, mercury

CAKUT Congenital abnormalities of the kidney and urinary tract, *PPAR-gamma* Peroxisome proliferator-activated receptor. Table was created by Drs Joseph Flynn and Nicholas Larkins

Consequently, aldosterone synthase activity is ectopically expressed in the adrenal fasciculata under control of ACTH rather than by ANG II or potassium. Aldosterone secretion becomes linked to cortisol secretion, and maintenance of normal cortisol levels results in constitutive aldosterone secretion, which leads to expanded plasma volume and HTN. The expanded plasma volume suppresses secretion of renin, but this fails to diminish aldosterone secretion.

Familial hyperaldosteronism type II (FH-II) is similar to GRA (FH-I), with excess production of mineralocorticoids, but is not suppressed by dexamethasone. FH-II may be associated with a family history of adrenal hyperplasia or adenoma and appears to be more common than FH-I. FH-II is autosomal dominant, and a recent study reported gain-of-function mutations in the *CLCN2* gene, encoding a chloride channel, ClC-2, located at chromosome 3q27 [61].

Familial hyperaldosteronism type III (FH-III) is characterized by massive adrenal hyperplasia with refractory HTN. Patients with the disease were found to have mutations in *KCNJ5* gene encoding the potassium channel Kir3.4 [62]. These mutations alter the ion selectivity of the channel, increasing its conductance to sodium. This results in depolarization of the glomerulosa cells, leading to increased calcium entry and aldosterone production.

Familial hyperaldosteronism type IV (FH-IV) is an autosomal dominant disease caused by gain-of-function mutations in the *CACNA1H* gene, located at chromosome 16p13.3 [63]. *CACNA1H* encodes a T-type calcium channel Cav3.2 localized to the zona glomerulosa. The mutations cause greater influx of calcium ions, stimulating aldosterone synthase.

Hypertension exacerbated in pregnancy (Geller syndrome) results from mutations in the mineralocorticoid receptor. It is an autosomal dominant form of HTN that is markedly accelerated in pregnancy [64]. A systematic screening of patients with early-onset HTN detected a missense mutation in the ligand-binding domain of the mineralocorticoid receptor MR S810L. Carriers of the mutation all developed HTN before age 20, a finding absent among their unaffected relatives. Steroids lacking 21-hydroxyl groups (progesterone, spironolactone) that normally bind but do not activate MR are all potent agonists of the mutant receptor. Because progesterone levels rise 100-fold during pregnancy, all pregnancies among patients harboring this mutation have been complicated by dramatic acceleration of HTN associated with complete suppression of the RAAS.

The syndrome of **apparent mineralocorticoid excess (AME)** is an autosomal recessive condition characterized by early-onset HTN with hypokalemia and metabolic alkalosis, accompanied by suppressed plasma renin activity and the virtual absence of circulating aldosterone. In AME, steroids other than aldosterone activate the mineralocorticoid receptor (MR). Affected patients have a lack-of-function mutation in the gene encoding the renal isoenzyme of 11-hydroxysteroid dehydrogenase (11HSD), resulting in impaired conversion of cortisol to cortisone. Although cortisol activates MR with potency similar to aldosterone, almost all activation of MR is mediated by aldosterone, despite its low plasma concentration. The specificity of MR for aldosterone in vivo is mediated by 11HSD activity, "protecting" the MR from cortisol by metabolizing it to cortisone, which does not activate MR. In AME, the absence of this enzyme allows cortisol to activate the MR, resulting in HTN mediated by increased sodium retention. The 11HSD-2 gene expressed in the principal cells of the cortical collecting duct shows homozygous loss-of-function mutations in AME patients. A systematic review of now 101 patients with the disease has been reported [65].

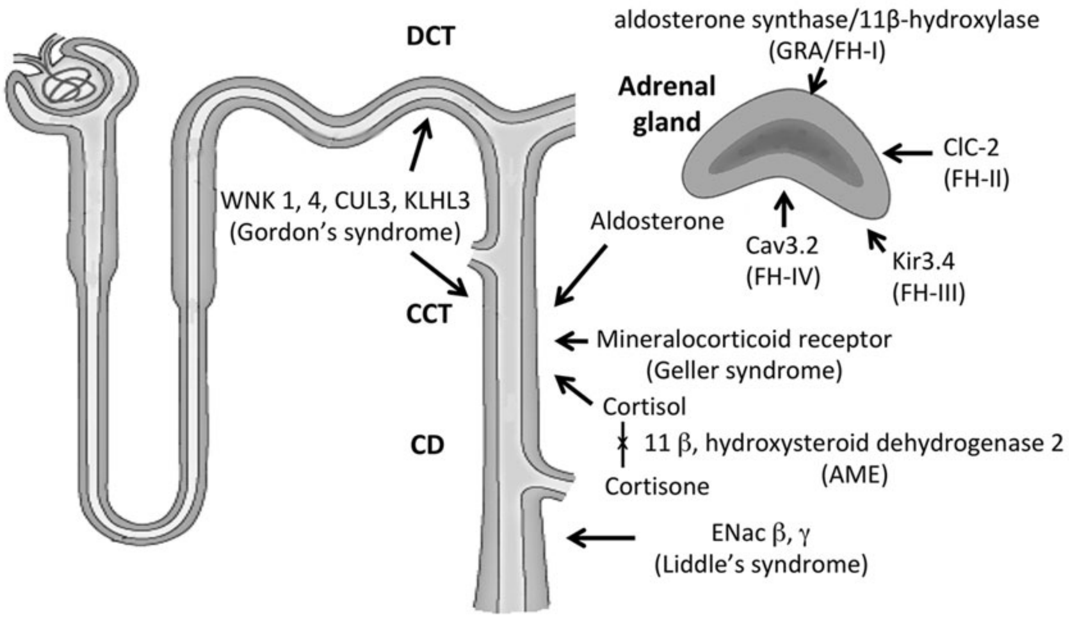

Fig. 4 Single-gene mutation causing hypertension. DCT, distal convoluted tubule; CCT, cortical collecting tubule; CD, collecting duct; GRA, glucocorticoid-remediable aldosteronism; FH, familial hyperaldosteronism; AME, apparent mineralocorticoid excess

Congenital adrenal hyperplasia results from two known defects in either 11-α-hydroxylase or17-α-hydroxylase activity and may cause HTN. These defects lead to overproduction of 21-hydroxylated steroids, which activate mineralocorticoid receptors, resulting in increased sodium reabsorption in distal tubules [60].

Primary glucocorticoid resistance (Chrousos syndrome) is a rare disease representing the partial resistance of the glucocorticoid receptor to glucocorticoids, resulting in excessive ACTH-dependent 11-deoxycortisone and corticosterone production. The disease is caused by a mutation in *NR3C1* gene encoding human glucocorticoid receptor located on chromosome 5q31.3. A cortisol level too high to be controlled by 11β-hydroxysteroid dehydrogenase type II enzyme activity activates mineralocorticoid receptors and increases sodium reabsorption in the distal tubules. Affected individuals have features of mineralocorticoid and androgen excess.

Liddle's syndrome is an autosomal dominant disorder characterized by severe HTN, metabolic alkalosis, and hypokalemia with low renin and aldosterone. Activating mutations in the β or γ subunit of the epithelial Na channel (ENaC), the main sodium channel responsible for sodium reabsorption in the renal collecting ducts, are responsible for constitutively elevated renal sodium reabsorption in Liddle's syndrome. Clearance of the normal channel from the membrane depends upon the sequence PPPXY in the cytoplasmic C termini of the β and γ ENaC subunits. Mutations of this sequence in either subunit prolong the cell surface half-life and increase the number of channels, by reducing channel ubiquitylation and internalization.

Pseudohypoaldosteronism type II, also known as Gordon's syndrome, is an autosomal dominant disorder characterized by HTN, hyperkalemia, hyperchloremic metabolic acidosis, and normal glomerular filtration rate. The WNK1 and WNK4 genes located on human chromosomes 12 and 17, respectively, are responsible for Gordon's syndrome. Two additional genes, CUL3 and KLHL3, located at chromosome 5q31.2 and 2q36.2, respectively, are involved in the proteasomal degradation of WNK proteins, and their mutations are also associated with Gordon's syndrome [66]. Deletions in the first intron of the WNK1 gene increase expression of the gene. This increases sodium reabsorption via ENaC and the Na^+/Cl^- cotransporter (NCC); the latter effect is caused by reducing inhibition of NCC by WNK4. Increased WNK1 expression also decreases potassium excretion by inhibiting the renal outer medullary potassium channel (ROMK). Missense mutations of the WNK4 gene have several effects on renal ion transport. In general, WNK4 inhibits NCC and prevents Na^+ reabsorption in the distal convoluted tubules. The mutated WNK4 does not inhibit NCC, leading to constitutively increased Na^+ reabsorption. WNK4 mutations also inhibit potassium excretion by ROMK, which contributes to hyperkalemia. Studies suggest that WNK1 may also play a role in the pathogenesis of salt-sensitive HTN.

Hypertension with brachydactyly features severe HTN with abnormal skeletal development in the hand and wrist. There are no associated distinguishing biochemical features. This syndrome has been mapped to chromosome 12p12.2 to p11.2 and caused by a mutation in the PDE3A gene encoding a phosphodiesterase [67]. A gain-of-function mutations decrease cellular cAMP levels leading to vascular smooth muscle cells proliferation, luminal narrowing of the vessels, and HTN.

Other Genetic Forms of Hypertension

Mutations in peroxisome proliferator–activated receptor γ (PPARγ) are dominant negative and lead to loss of function. These mutations result in insulin resistance, type II diabetes, and HTN at an early age. Another syndrome of HTN, hypercholesterolemia, and hypomagnesemia shows maternal inheritance of a homoplasmic mutation caused by a cytidine substitution in the mitochondrial tRNA. Mutated tRNA may lead to defective nucleotide modifications, altering structure and function of mitochondria [68].

Hypertension in Renovascular Disease

Readers are referred to ▶ Chap. 64, “Renovascular Hypertension in Children: Evaluation and Management.”

Hypertension in Acute Kidney Injury and Chronic Kidney Disease

Readers are referred to ▶ Chaps. 67, “Evaluation and Management of Acute Kidney Injury in Children,” and ▶ 70, “Epidemiology and Management of Chronic Kidney Disease in Children.”

Hypertension in Dialysis Patients

Readers are referred to ▶ Chaps. 75, “Peritoneal Dialysis,” and ▶ 76, “Chronic Hemodialysis in Children.”

Hypertension After Renal Transplantation

Readers are referred to Chap. “Renal Transplantation.”

Hypertension in Coarctation of the Aorta

Coarctation of the aorta (CoA) occurs in approximately 1 out of 2500 live births and accounts for 5–10% of all congenital heart disease. CoA-associated HTN has been described in three clinical settings: prior to surgical repair, in the immediate postoperative period following surgical repair, and years after repair. Pre-repair HTN is the result of renal hypoperfusion and activation of the RAAS. There may also be increased SNS activity. In the immediate postsurgical period, paradoxical HTN may be seen, probably resulting from increased activity of the RAAS and SNS. Despite surgical repair of CoA, many patients will later be found to have abnormal BP at rest. Ambulatory BP monitoring (ABPM) usage is recommended to detect HTN in this population. Patients with CoA repair were found to have vascular dysfunction such as reduced arterial response to glycerltrinitrite and impaired flow-mediated dilatation. Carotid intima-media thickness and pulse wave velocity were also significantly increased. These vascular dysfunctions affect BP control and ultimately increase the risk of cardiovascular events. Impaired baroreceptor sensitivity also has been shown to be a key for HTN following CoA repair. These findings suggest that vascular dysfunction may persist regardless of the repair and lead to HTN once the compensation fails. Recoarctation should also be investigated in late post-repair HTN.

Hypertension in Vasculitis

Because of the numerous vessels within the renal parenchyma, the kidneys are frequently targeted by systemic vasculitis. Glomerular inflammation with cellular infiltration, proliferation, and immune complex deposition resulting in nephritis and renal failure has been well characterized in systemic lupus erythematosus (SLE), microscopic polyarteritis, granulomatosis with polyangiitis, IgA vasculitis, and cryoglobulinemic vasculitis. Takayasu's arteritis (TA), an inflammatory process associated with stenosis and obliteration of the aorta and its primary branches, induces HTN by mechanisms similar to those seen in CoA, renovascular HTN, or ischemic kidney disease, as discussed above. Necrotizing vasculitis, such as classic polyarteritis nodosa and Kawasaki's disease, may lead to HTN secondary to thrombosis of inflamed major extrarenal and intrarenal arteries with resulting local (intrarenal) RAAS activation and infarction of the renal parenchyma.

Hypertension in Endocrine Diseases

HTN can occur secondary to many hormonal derangements, including excess production of mineralocorticoids, glucocorticoids, and catecholamines. An excess of growth hormone or parathyroid hormone, hypo- or hyperthyroidism, a renin-secreting tumor, or diabetes mellitus (DM) may also lead to HTN [69].

Pheochromocytomas and paragangliomas are catecholamine-secreting tumors. Pheochromocytomas arise from the adrenal medulla, and paragangliomas arise from extra-adrenal sites, mostly in the head and neck, and also in the thorax, abdomen, pelvis, and bladder. Sustained or paroxysmal HTN is the most common clinical sign of pheochromocytoma, although this may be less common in children. Pheochromocytomas in children have a higher incidence of bilateral adrenal tumors, multiple tumors, and extra-adrenal tumors. The incidence of malignant pheochromocytomas and paragangliomas is higher in children and adolescents.

Although most pheochromocytomas are sporadic, there is a familial predisposition in patients with multiple endocrine neoplasia (MEN) type II, von Hippel–Lindau disease, neurofibromatosis type I, and familial paraganglioma. Rarely, phenochromocytomas/paragangliomas may be found in tuberous sclerosis, Sturge-Weber syndrome, and ataxia-telangietasia. Pheochromocytoma in MEN type II is associated with a germline mutation of the proto-oncogene RET, and pheochromocytoma in von Hippel–Lindau disease results from germline mutation of the *VHL* gene [70]. Familial paraganglioma is an autosomal dominant disorder with catecholamine HTN.

In addition to the genes mentioned above, mutations of succinate dehydrogenase subunit genes (*SDHB, SDHC, SDHD, SDHAF2, and SDHA*), a part of the mitochondrial complex, are found. Mutations of the *TMEM127* gene, which acts as a negative regulator of the mTOR signaling pathway, and mutations of the *MAX* gene, which affects cell proliferation, differentiation, and apoptosis, are associated with pheochromocytomas/paragangliomas.

HTN in pheochromocytoma results from high circulating levels of catecholamines, primarily norepinephrine, epinephrine, and dopamine. Some tumors produce the catecholamine precursor L-dopa. Pheochromocytomas also secrete numerous other peptide hormones, including renin, VIP, neuropeptide Y, somatostatin, and ET-1. These substances are responsible for many of the nonhypertensive symptoms including flushing, sweating, and diarrhea.

Primary hyperaldosteronism (PAL) is now believed to be much more common than previously thought [71]. PAL was first reported by Conn as aldosterone-producing adenoma (APA) and commonly results from adrenal hypertrophy ("idiopathic hyperaldosteronism, IHA"). Rarely, pure aldosterone-producing adrenocortical carcinoma and ectopic aldosterone-secreting tumors cause PAL. GRA, FH-II, FH-III, and FH-IV, which were previously described in this chapter, are also in this category.

APA and IHA both cause excess aldosterone secretion, and both produce HTN with similar clinical features: hypokalemia, renal potassium wasting, suppressed plasma renin activity, and increased plasma and urinary levels of aldosterone. The BP elevation due to aldosterone excess results from an increase in SVR due to aldosterone-mediated increased plasma sodium concentration, which leads to a rise in the intracellular calcium. The increase in intracellular calcium leads to impaired left ventricular diastolic relaxation and enhanced contractility with reduced vascular compliance. Recurrent somatic mutations of the *KCNJ5* gene encoding the potassium channel Kir3.4 may account for 40% of the APA patients, and a germinal *KCNJ5* mutation may be associated with FH-III.

Cushing's syndrome or glucocorticoid excess is caused by exogenous corticosteroids or hypersecretion of corticotropin (ACTH) due to pituitary tumors, ectopic ACTH secretion, or adrenocortical tumors (REFS). There are several mechanisms that contribute to the HTN in Cushing's syndrome [72]. The 11β-HSD2 is overwhelmed in the setting of hypercortisolism, and cortisol binds the mineralocorticoid receptor leading to sodium retention, potassium excretion, and increased blood volume. Hepatic synthesis of angiotensinogen, which stimulates the RAAS, is activated. Upregulation of catecholamine production in the adrenal chromaffin cells and increased peripheral vascular sensitivity to adrenergic agonists may also play a role in the HTN. Furthermore, cortisol affects the vascular system by decreasing vasodilatory elements such as nitric oxide synthase, prostacyclin, prostaglandins, and kallikreins and increasing vasoconstricting ET-1.

Acromegaly or growth hormone (GH) excess. The prevalence of HTN in acromegaly is more than 50% [73]. Excess GH leads to insulin resistance and stimulates smooth muscle cell growth and HTN [73]. Impaired NO production and sodium retention with volume expansion also play a role.

Primary hyperparathyroidism (PHPT). PHPT is strongly correlated with HTN with the prevalence ranging from 47% to 63%. Potential pathogenic mechanisms of HTN in PHPT are elevated intracellular calcium, hypomagnesemia, increased plasma renin activity, and glucose abnormalities. Impairment of endothelial vasodilation by increased intracellular calcium and vasospasm induced by neurohormonal substances due to hypomagnesemia have been reported.

Thyroid disorders. Hypothyroidism as the cause of HTN is often overlooked [74]. Diastolic HTN results from compensatory mechanisms – primarily increased peripheral resistance mediated by increased responsiveness to adrenergic stimulation and increased SNS activity. Also, renin release is decreased and salt sensitivity is increased, leading to increased sodium reabsorption and volume expansion.

Hyperthyroidism, on the other hand, is associated with activation of the RAAS secondary to systemic vascular resistance reduction [69]. Enhanced sodium reabsorption leads to volume expansion. Arterial stiffness is increased despite low systemic vascular resistance leading to a widened pulse pressure. There is also ß2 receptor stimulation, resulting in vasodilatation and increased cardiac output and contractility. The net effect is isolated systolic HTN.

Reninoma. Juxtaglomerular cell tumors of the kidney or ectopic tumors secrete renin and induce HTN associated with hypokalemia, hyponatremia, and other features of RAAS activation. There are approximately 100 reported cases and common in adolescents and young adults [75]. It is also twice as common in women.

Diabetes Mellitus. HTN in patients with type I DM is usually correlated with the development of microalbuminuria and diabetic nephropathy. On the other hand, the majority of patients with type II DM have HTN prior to the development of nephropathy. The pathogenesis of HTN in DM is multifactorial, with hyperinsulinemia, sodium retention, volume expansion, and increased arterial stiffness all playing important roles. Hyperglycemia alters lipid and protein metabolism, and insulin increases SNS activity. DM leads to both microangiopathic and macroangiopathic vascular changes, and autonomous neuropathy causes vascular dysfunction. Numerous studies have shown that endothelial dysfunction in DM may play a significant role in HTN by altering vascular architecture and remodeling in response to hemodynamic changes, low NO and prostacyclin release, increased oxygen-reactive species, and increased ET-1. Insulin may stimulate vascular smooth muscle proliferation and increase peripheral vascular resistance. Hyperglycemia and advanced glycosylation end products may also be important contributing factors.

Hypertension in Pulmonary Diseases (BPD, Sleep Apnea)

HTN in children with chronic lung disease is most commonly seen in infants with bronchopulmonary dysplasia (BPD). An increase in carotid and brachial artery stiffness was associated with a neonatal history of moderate to severe BPD and higher adult diastolic BP [76]. Because the development of HTN is correlated with the severity of lung disease, chronic hypoxia may also be an important mechanism.

Obstructive sleep apnea (OSA), characterized by episodic upper airway obstruction during sleep despite persisting respiratory efforts, leads to intermittent asphyxia, concomitant hypoxia and hypercapnia, and disruption of sleep. As mentioned in primary HTN section above, OSA is a risk factor for HTN. The cycles of hypoxia and hypercapnia result in stimulation of peripheral chemoreceptors and increased sympathetic outflow. Other important contributing factors include endothelial dysfunction, oxidative stress, and inflammation [77]. Because many patients with OSA are obese, coexisting conditions such as metabolic syndrome are probably also involved in the development of HTN.

Hypertension in Neurologic Disorders

Systemic HTN can be seen in a variety of neurologic disorders, including stroke, spinal cord injury, seizures, poliomyelitis, hydrocephalus, head trauma, psuedotumor cerebri (idiopathic intracranial HTN), Guillain-Barre syndrome, and with space-occupying central nervous system lesions (tumor, bleeding, and abscess). Many of these are associated with increased sympathetic outflow, leading to peripheral vasoconstriction and renal sodium retention (see earlier sections). Spinal cord injury, usually above T6, may induce HTN due to aberrant sympathetic activity below the lesion. Widespread SNS activation can be elicited by noxious or non-noxious stimuli, a phenomenon called autonomic dysreflexia. Increased levels of circulating renin and catecholamines have also been reported. In familial dysautonomia, HTN is attributed to central autonomic dysfunction, sympathetic and parasympathetic efferent baroreflex dysfunction, or afferent baroreflex failure.

Increased intracranial pressure (ICP) causes systemic HTN that can be quite labile. ICP is a reversible determinant of efferent sympathetic outflow and the existence of a possible intracranial baroreflex is suggested [78]. Circulating levels of catecholamines may be increased. Hemodynamically, HTN is primarily due to increased SVR. In many instances, especially when ICP is elevated due to trauma, systemic HTN is an important compensatory mechanism for the maintenance of normal cerebral perfusion.

Pregnancy-Induced Hypertension and Preeclampsia

Pregnancy-induced HTN (PIH) may affect up to 8% of all first pregnancies in the USA. PIH includes gestational HTN, preeclampsia, and chronic HTN with superimposed preeclampsia. Preeclampsia is new-onset HTN and new-onset end-organ damage, including proteinuria, after 20 weeks of gestation [79]. The pathophysiology of this complex process involves multiple organ systems. Abnormal cytotrophoblast invasion of spiral arterioles leading to reduced utero-placental perfusion and ischemia appears to be the initiating event. Placental ischemia results in activation/dysfunction of the maternal vascular endothelium, which increases synthesis and release of vasoactive factors such as soluble Fms-like tyrosine kinase-1 (sFlt-1), cytokines, and Ang II type 1 receptor autoantibodies (AT_1-AA).

Increased production of sFlt-1 triggers an imbalance between pro-angiogenic factors (VEGF and PlGF) and anti-angiogenic factor (sFlt-1). This imbalance reduces active angiogenesis, which is necessary for development of the placental vascular network. Another anti-angiogenic protein, soluble endoglin (sEng), may interfere with transforming growth factor β-1 binding to receptors on endothelial cells, which may reduce endothelial NO and vasodilatation. Ischemia of the placenta also increases thromboxane and AT_1-AA, leading to increased production of reactive oxygen species and ET-1, and decreases formation of vasodilators such as NO and prostacyclin. There is also increased synthesis of inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and IL-6, which is activated by TNF-α. Those cytokines, in turn, induce structural and functional alterations in endothelial cells, enhance endothelin production, and reduce acetylcholine-induced vasodilatation.

Another important mechanism may be defective NO production. During normal pregnancy, maternal CO and blood volume increase by 40–50%, whereas SVR and BP tend to decrease. In addition, GFR and renal plasma flow increase by 30–40%. There is substantial evidence that NO plays an important role in mediating those changes. Plasma and urinary levels of cGMP, a marker of NO bioavailability, increase during pregnancy in rats, and urinary nitrate/nitrite excretion has been reported to increase. NO synthase inhibition in pregnant rats produces HTN associated with peripheral and renal vasoconstriction, proteinuria, intrauterine growth retardation, and increased fetal morbidity, closely resembling the symptoms of human PIH. Iimmunological factors could also contribute to preeclampsia including HLA class I antigens (HLA-A, HLA-E, and HLA-G), helper T cells, natural killer cells, and complements.

Drug-Induced Hypertension

Numerous therapeutic and illicit drugs, as well as some commonly used dietary supplements, may influence BP significantly, especially in individuals whose BP regulation is already disturbed. A detailed discussion of the many substances with effects on BP would be beyond the scope of this chapter.

Normative Blood Pressure Values in Children and Adolescents

Casual Blood Pressure Measurement

In 2017, the American Academy of Pediatrics (AAP) published its first clinical practice guidelines (CPG) for screening and management of BP in children and adolescents [80]. This was an update to the 2004 guidelines previously published by the National High Blood Pressure Education Program Working Group in the USA [81]. There were several key updates in the 2017 AAP CPG regarding casual BP measurement. First of these updates was that for adolescents 13–17 years old, the BP category threshold values were no longer the percentile based, but instead matched those of 2017 American Heart Association (AHA) and American College of Cardiology adult HTN guidelines. Second, the normative BP tables based on age, gender, and height were revised to include only the auscultative BP measurements from normal-weight children rather than the previous normative data that included overweight and obese children. Third, the threshold value definition for stage 2 HTN for 1- to 12-year-old children changed from ≥99th percentile +5 mm Hg to ≥95th percentile +12 mm Hg. Lastly, the category named "prehypertension" was replaced by "elevated BP."

The most recent European pediatric guidelines were published in 2016 by European Society of Hypertension (ESH) [82]. Some of the unique aspects these guidelines with regards to casual BP include percentile-based BP categories for 0- to 15-year-olds, and adult definitions and threshold values for ≥16-year-olds, a category called "high normal" to mirror "elevated BP" category, and a distinct category called "isolated systolic HTN." The ESH guideline uses the same data source as the US 2004 guidelines, and stage 2 HTN threshold value for 0- to –15-year-olds remained ≥99th percentile +5 mm Hg. ▶ Chap. 63, "Evaluation and Management of Hypertension in Children" provides more detailed discussion on definition, categories, standards, and other aspects of casual BP measurements.

Ambulatory Blood Pressure Monitoring

Ambulatory BP monitoring (ABPM) is a more comprehensive method to evaluate BP in both adults and children and is most often performed for a 24-h period. Advantages of ABPM over casual BP include reproducibility, better correlation with hypertensive target organ changes, and cost effectiveness [83]. ABPM is optimal in diagnosing children with white coat HTN (WCH) and masked HTN (MH) [83]. The 2017 AAP CPG expanded the recommended uses of ABPM to include A) confirmation of HTN after casual BP measurements in the elevated BP category for 1 year or with stage 1 HTN readings over three clinic visits, B) assessment of HTN severity in children and adolescents with high-risk conditions and determine if abnormal circadian BP patterns are present, C) evaluation for suspected WCH, D) detection of HTN (including MH) in patients who have undergone coarctation repair, E) assessment of treatment effectiveness especially when clinic and/or home BP measurements indicate insufficient BP response to treatment, and F) monitoring of CKD with HTN patients to lower 24-h mean arterial pressure (MAP) <50th percentile [80].

ESH also endorses the use of ABPM in pediatrics in similar clinical scenarios and in high-risk patients such as DM, CKD, solid organ transplantation, severe obesity with or without sleep-disordered breathing, and assessment of HTN treatment efficacy. In addition, ESH guidelines recommend its use in children with hypertensive response during treadmill test, autonomic dysfunction, suspicion of catecholamine secreting tumors, and in clinical trials [82]. Parallel to casual BP measurements, ABPM threshold values to define BP categories are statistical based as discussed further below. The normative ambulatory BP values were derived from 1141 all Caucasian German children and adolescents and later reanalyzed by using the least mean square method to create the normative table [84].

The major disadvantages of current ABPM practice include lack of availability of ABPM across the world especially in low- to middle-income countries, and limitation of normative BP tables due to the limited data for small children and lack of ethnic and racial diversity of the normative data.

Home Blood Pressure Measurement

While ABPM has many advantages, monitor availability for pediatricians around the world is a major limitation. In adults, self-measurement of home BP is regarded as an alternative and has been recommended in clinical practice guidelines [85]. Like many ambulatory BP monitors, home monitors are oscillometric devices. There is pediatric data for home BP measurements including reproducibility, validation, and reference values [86]. Using ABPM as the reference method, sensitivity, specificity, and positive and negative predictive values of home BP measurements for the diagnosis of HTN were 55, 92, 74, and 82%, respectively; for the diagnosis of WCH 89, 92, 70, and 98%, respectively; and for the diagnosis of MH 36, 96, 50, and 93%, respectively [87].

Clinical practice guidelines for home BP monitor usage differs between the latest ESH and AAP guidelines. The ESH endorses its usage [82], while the AAP CPG recommends avoidance of home BP monitoring for diagnosis of HTN, WCH, and MH, while they endorse its use as an adjunct to casual BP and ABPM after HTN diagnosis is established [80]. Website https://www.stridebp.org/bp-monitors maintains an updated list of pediatric validated home BP monitors.

Definition of Hypertension in Children

Casual Blood Pressure Measurement

In contrast to adults, children and adolescents rarely suffer from hypertensive cardiovascular events, and HTN definition threshold values are statistically defined rather than outcome based. Diagnosis of HTN is made only if a child or adolescent has auscultatory confirmed BP readings ≥95th percentile on at least three different visits [80, 82]. The 2017 AAP CPG endorses the use of pediatric validated automated oscillometric devices for casual BP measurement, but when abnormal BP is detected (≥90th percentile or >120/80), confirmation by average of two auscultatory BP measurement is recommended to define the BP category [80]. As mentioned above, one of the major updates of the 2017 AAP CPG was the exclusion of BP measurements from overweight and obese healthy children for its data source to create the new normative tables [80]. This resulted in reduction of 2–3 mmHg for corresponding percentiles for most younger children. Another update of the 2017 AAP CPG is the use of adult BP categories for adolescents ≥13 years old [80]. ESH guideline recommends the use of adult BP categories for adolescents ≥16 years old [82]. Implication of these definition changes in adolescents is the increase of respective threshold values, especially in shorter adolescent females. For example, the 95th percentile systolic BP for a 16-year-old female with fifth percentile height is 124 mmHg, but in the newer guidelines, the threshold value for stage 1 HTN is now higher at 130 mmHg.

Ambulatory Blood Pressure

ABPM provides additional BP data such as mean systolic BP, mean diastolic BP, and MAP for the entire 24 hours, awake hours, and sleep hours. Both AHA and ESH recommend the use of the 95th percentile values ("gender and age" or "gender and height" tables are available) from Wühl et al. [84] as the threshold values to define HTN. ESH guideline also specifies the use of adult threshold values (24 h: 130/80 mmHg, awake 135/85 mmHg, sleep 125/75 mmHg) if the 95th percentile values are higher than these adult threshold values [82]. In addition, ABPM provides "BP load," defined as the percentage of BP readings exceeding the threshold values, and "nocturnal dipping," defined as the percentage difference between awake and sleep mean BP values ([mean awake BP – mean sleep BP]/mean awake BP x 100). The clinical significance of BP load is that higher load values may be associated with left ventricular hypertrophy [88], and BP load is now part of the staging schema in the most recent AHA ABPM scientific statement [83]. This statement suggests the use of casual BP and ABPM data, including mean awake and sleep SBP and DBP, and awake and sleep SBP and DBP loads, to determine BP categories of normal BP, WCH, prehypertension, MH, ambulatory HTN, and severe ambulatory HTN. Normal nocturnal dipping is defined as ≥10%, and blunted nocturnal dipping is defined as 0–10%, and reverse dipping is defined as mean sleep BP higher than mean awake BP. Abnormal nocturnal dipping has been associated with microalbuminuria in children with type 1 DM [89], and ABPM use to evaluate nocturnal BPs are recommended in high-risk patients such as those with DM, CKD, solid organ transplant, and severe obesity [83]; the current guidelines, however, do not include nocturnal dipping as part of the aforementioned BP staging schema.

Lastly, although MAP is currently not part of the current BP staging schema, one pediatric study reported an increase of HTN detection by 19% when MAP was used in the definition of HTN, and the authors suggested the inclusion of MAP in the definition of ambulatory HTN since most ABP monitors are oscillometric devices and actually measure MAP rather than systolic and diastolic BP [90]. There is one specific clinical scenario when 24-h MAP from ABPM is of clinical use currently. The ESCAPE trial demonstrated that tighter BP control defined by 24-h MAP <50th percentile resulted in slower decline in renal function compared to MAP in the 50th–95th percentile range in pediatric CKD patients [91]. And therefore, the 2017 AAP CPG recommends the use of ABPM in children and adolescents with CKD and HTN to guide treatment to achieve 24-h MAP <50th percentile [80].

Epidemiology of Hypertension

Prevalence of Hypertension in General Population

If the 95th percentile from the normative table is used as threshold values, by definition, 5% of children will be diagnosed as hypertensive. However, BP measurements obtained to formulate the normative data were from first time visits for each child. In clinical settings where repeat BP measurements take place, the prevalence of confirmed HTN is lower partly due to inherent BP measurement variability, accommodation effect, and tendency for regression toward the mean phenomenon. There have been many pediatric HTN prevalence studies around the world, but comparison among different regions is difficult due to nonstandardized methods to diagnose HTN. However, Song et al. screened 9084 records and performed meta-analysis of 47 articles from around the world that used the 2004 Fourth report BP definitions and at least three separate BP measurements. Table 3 summarizes some of the main findings from this meta-analysis. Overall global pediatric prevalence rate of HTN was 4% with an additional 9.7% with prehypertension (equivalent to "elevated BP" or "high normal") [92]. World Health Organization–defined region-specific rates were as follows: Africa 6.9%, Americas 3.0%, Eastern Mediterranean 5.3%, European 4.1%, Southeast Asia 3.1%, and Western Pacific 4.6% with no statistical differences between these regions. There were also no statistical differences in prevalence rates based on gender, and urban versus rural settings. There was a clear pattern of increasing prevalence rates of HTN with increasing body mass index group with normal weight group prevalence rate of 1.9%, overweight group rate of 5%, and obese group rate of 15.3%. Perhaps most concerning finding is the clear pattern of increasing prevalence rates over the years: prevalence rate in the 1990s was 1.3%, 2000s was 3.3%, and 2010–2014 was 6%.

Following the same trajectory, more recent US studies have reported HTN prevalence of approximately 3.5% and "prehypertension" of approximately 2–3.5% [93, 94], an increase from earlier reports of around 1% or lower. As for abnormal BP measurements (both HTN and prehypertension categories) based on single measurements, the US National Health and Nutrition Examination Survey has reported 15–19% for boys and 7–12% for girls with higher rates among Hispanic and non-Hispanic African Americans compared to non-Hispanic Caucasians [95].

Multiple European studies have reported prevalence rates with central Europe having similar rates as the USA, and southern European studies demonstrating higher rates. Adolescent HTN prevalence rates were reported as 2.2% in Switzerland, up to 4.9% in Poland [94, 96], and in Greece and Portugal, they were in the range of 12–14% [97, 98].

At this time, there has not been any new prospective studies to report the updated pediatric HTN prevalence based on the 2017 AAP CPG definitions, but several publications have addressed potential implications mostly by cross-sectional study design. These studies had different subject selection and population: the US NHANES cohort, Chinese school-based screening, and high-risk adolescents. They showed modest increase in prevalence of high BP measurements and HTN of approximately 1–5% when applying the different definitions from the 2017 AAP CPG versus 2004 Fourth Report [99–102]. These studies also noted an increase in the likelihood of upward reclassification of BP categories for males, and obese participants. The 2017 AAP CPG definitions were also applied to American school–based screening program data. The prevalence of high BP increased by 1.5% (16.3 versus 14.8%) when applying the 2017

Table 3 Pediatric hypertension and prehypertension prevalence rates around the world: global overall and World Health Organization designated regions

	Hypertension (%)	Prehypertension (%)
Overall	**4.00**	**9.41**
Africa	6.94	
Americas	3.02	9.79
Eastern Mediterranean	5.26	
European	4.09	9.08
Southeast Asia	3.10	
Western Pacific	4.64	

BP category definition from the 2004 Fourth report. Data are extracted from reference [141]

AAP CPG compared to the 2004 Fourth Report, but the overall combined stage 1 and 2 prevalence of HTN decreased slightly from 2.7% to 2.3% [103]. The authors hypothesized that these reclassifications were likely due to a combination of lowering BP threshold values for most of the <13-year-old cohort and implementation of the standard adult threshold value of 130/80 for ≥13-years-old cohort, which had either increased or decreased threshold values depending on adolescents' age, gender, and height.

Lastly, incidence of pediatric HTN data is limited due to challenges of long-term longitudinal follow-up with requirement of multiple BP measurements. Redwine et al. reported overall incident rate of 0.5%/year from a multiethnic American school–based BP screening program of ages 10–19 years old using the 2004 Fourth report BP definitions, with higher rates of incidence if they were originally in the category of "at-risk" or "pre-hypertension" [104]. This rate may sound small, but in a population of 100,000 adolescents, this incident rate would translate to 1500 adolescents with new HTN diagnosis in 3 years.

Prevalence of Hypertension in Special Population

There are numerous pediatric chronic diseases where HTN prevalence rates have been reported to be significantly higher compared to the healthy general population with a number of these populations discussed in this section. From a large American multicentered observational cohort study, Chronic Kidney Disease in Children study (CKiD) where 69% of the cohort was on antihypertensive medication at the time of ABPM, HTN was diagnosed by ABPM in 52% [105]. Of these hypertensive CKD children, 37% were diagnosed to have MH, and only 4.1% were diagnosed to have WCH. This CKiD cohort also demonstrated that proper diagnosis and treatment of HTN is of the essence as there were significantly higher rates of LVH in hypertensive CKD patients compared to normotensive CKD patients [106].

In children who had successful coarctation repair at a median age of 24 months, in a cross-sectional study with a median age of 14.5 years old, 45% of the cohort was found to have MH by ABPM [107].

In DM where early onset of CVD is of significant concern, rates of HTN appear to be higher in children and adolescents with type I and II DM. In an European single center cross-sectional analysis of type I DM patients with a median age of 14.9 years old, 51% had hypertensive BP measurement (≥95th percentile), and 29% had ABPM confirmed HTN [108]. In an American multicentered multiethnic longitudinal follow-up of type II DM adolescents where all participants had <2 years of diabetes diagnosis duration, HTN prevalence was 12% at baseline, and 34% by end of study 3.9 years later [109].

In the solid organ transplant population, post-kidney transplant cohort has been most frequently studied. Common HTN risk factors in solid organ transplantation recipients include the use of immunosuppressive medications known to raise BP, such as corticosteroids and calcineurin inhibitors, and an increase in the risk of metabolic syndrome, and various degrees of CKD. In post-kidney transplant children and adolescents, the prevalence of HTN has been reported to range between 60% and 90%, with LVH reported in 50–80%, and relatively poor control of HTN with only 20–50% of treated children reaching normal BP [110]. The data is more scant for non-kidney solid organ transplantation. Registry of pediatric post-liver transplantation reported elevated BP measurement (≥95th percentile or on antihypertensive medication) prevalence of 17.5–27.5% during their annual follow-up between years 5 and 10 post-transplant [111]. According to the registry of the International Society for Heart and Lung Transplantation, nearly 70% of pediatric heart transplant recipients were diagnosed with HTN at 5 years post-transplant [112].

Finally, there are genetic conditions known to be associated with HTN including Turner syndrome and neurofibromatosis type I. HTN is a well-known major risk factor for CVD in adult women with Turner syndrome, but very little pediatric prevalence data is available. In a single-centered British study with 62 females aged 5–22 years old, 24-h ABPM found 21% of patients with mean systolic, and 17% with mean diastolic measurements above the 95th percentile for age and gender [113]. In a single-centered Italian study with 27 patients aged 4–24 years old and followed for 2–10 years at least annually for neurofibromatosis type II, the HTN prevalence rate was 19% [114].

Prevalence of White Coat Hypertension

There have been several studies that have reported prevalence of pediatric WCH with widely varied percentages partly due to changing casual BP category definitions and ABPM BP staging schema over time. Earlier US studies with different ABPM definitions reported the prevalence of WCH to be around 50% of referred patients for elevated BP [115, 116], but these studies did not use BP load data from ABPM as part of the BP staging schema. Another ABPM study demonstrated that when BP load was included in the diagnostic schema, rates of WCH decreased from 45% to 22% [117].

Adult WCH outcome data show mixed results, and WCH may represent a risk factor for eventual progression to sustained HTN [118] and may serve as an intermediate risk factor for both hypertensive target organ damage and cardiovascular events [119]. Pediatric WCH data is even more scant. One small study in Sweden demonstrated that 23% of children initially diagnosed with WCH progressed to sustained HTN with median follow-up time of approximately 9 year, although obesity may have played a major contribution [120]. This study also demonstrated that pulse wave velocity and carotid intimal-medial thickness were significantly higher in those who progressed to sustained HTN compared to those who remained as WCH, while there was no difference in left ventricular mass index. As for target organ changes, the risk for left ventricular hypertrophy in WCH is not increased compared to normotensive children, but left ventricular mass index may be intermediate between normotensives and sustained hypertensives after controlling for body mass index [115, 121].

Prevalence of Masked Hypertension

MH is the converse of WCH where patients have below the threshold BP readings in a clinic-based setting but have above-threshold readings in out-of-clinic settings. In the adult population, MH prevalence has been estimated to be ~9–17%, and the cardiovascular risk associated with MH approaches the risk associated with sustained HTN [122]. Pediatric studies have reported prevalence of ~8–17% in healthy children [123, 124], and 9% among children referred for BP evaluation [125]. Risk factors for MH in these studies include obesity and family history of HTN. Although no pediatric data exist for cardiovascular events with MH, these studies have also demonstrated increased left ventricular mass index in children and adolescents with MH [123–125]. In children and adolescents with chronic conditions, including those after coarctation or aorta repair, CKD, post-solid organ transplantation, and sickle cell disease, MH has been reported to be much higher [105, 107, 126–128]. And thus, the most recent AHA pediatric guideline recommends routine use of ABPM for evaluation of MH in a) clinical suspicion of HTN but normal or elevated BP clinic measurements, b) DM, c) CKD, d) solid organ transplant, and e) severe obesity with and without sleep-disordered breathing [83].

Conclusion

During the decades, there has been increasing interest and progress in the field of HTN in children and adolescents. Emerging new techniques may provide insights into the pathophysiology of HTN and generate new hypothesis. Future epidemiological studies should aim at defining HTN in relation to target organ damage. Large population-based studies are also needed to investigate whether various intervention methods prevent development of HTN and CVD in the adulthood.

Cross-References

- ▶ Chronic Hemodialysis in Children
- ▶ Epidemiology and Management of Chronic Kidney Disease in Children
- ▶ Evaluation and Management of Acute Kidney Injury in Children
- ▶ Peritoneal Dialysis
- ▶ Renovascular Hypertension in Children: Evaluation and Management

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Evaluation and Management of Hypertension in Children

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Abstract

Hypertension is an increasingly common and important problem in children. This chapter provides a clinical approach to childhood hypertension based on current evidence. Frameworks for defining and classifying hypertension are provided in the context of two main international guidelines, produced by the American Academy of Pediatrics and European Society of Hypertension. Included are recommendations about how best to measure BP and the role of ambulatory blood pressure monitoring.

The approach to evaluating hypertension continues to evolve, reflecting changes in test accuracy and the pretest probability of secondary hypertension. Available tests and indications for ordering these are discussed in the context of presenting signs and symptoms. Both non-pharmacologic and pharmacologic therapies are covered. Dietary interventions and exercise are both utilized in all children with hypertension, but particularly in the setting of obesity. The rationale and thresholds for pharmacologic therapy are discussed, along with recommendations about the treatment of children with secondary or severe hypertension. The chapter concludes with discussion of unanswered research questions and needs.

Keywords

Blood pressure · Primary hypertension · Monogenic hypertension · Secondary hypertension · Left ventricular hypertrophy · Ambulatory blood pressure monitoring

Introduction

The prevalence of hypertension (HTN) among children is increasing, driven by an increase in primary HTN, observed in conjunction with the global obesity epidemic [1–3]. While the principles of diagnosis and management have not changed substantially, they have shifted to better include children with primary HTN while ensuring that children with secondary HTN are not missed.

The most commonly used guidelines are those from the American Academy of Pediatrics (AAP) and European Society of Hypertension (ESH) [4–6]. As reviewed below, there are some key differences between the two guidelines, but they largely agree regarding the methods used to diagnose and treat children with HTN.

Definitions and Classification

Blood pressure (BP) levels used to define HTN in adults have recently been revised [7, 8]. They are based on the results of long-term studies that have demonstrated an increase in the number of adverse events such as myocardial infarction, stroke, and sudden cardiac death with higher BP levels. Given that these events occur only rarely in the pediatric population, it is not possible at present to define childhood HTN based on outcomes. Therefore, a statistical approach has been adopted that defines abnormal BP levels in children and adolescents according to the distribution of BP in healthy children.

The BP classification schemes of the AAP and ESH pediatric guidelines are summarized in

Table 1 Blood pressure categories in AAP and ESH guidelines

European guidelines			US guidelines		
	<16 years	≥16 years		<13 years	≥13 years
Normal BP	<90th %ile	<130/85	Normotension	<90th %ile[a]	<120/80
High-normal BP	≥90th %ile–<95th %ile	130–139/85–90	Elevated BP	≥90th %ile[a]–<95th %ile	120–130/80
Grade I HTN	≥95th %ile–99th %ile + 5 mmHg	140–159/90–99	Stage I HTN	≥95th %ile–<95th %ile +12 mmHg[b]	130/80–139/89
Grade II HTN	>99th %ile + 5 mmHg	160–179/100–109	Stage II HTN	≥95th %ile + 12 mmHg[c]	≥140/90
Isolated systolic HTN	SBP>95th % ile and DBP <90th %ile	SBP >140 and DBP<90	Isolated Systolic HTN	Not addressed	Not addressed
Immediate referral to emergency care	Severe HTN[d] associated with life-threatening condition	Severe HTN[d] associated with life-threatening condition	Immediate referral to emergency care	>95th %ile + 30 mmHg[e]	>180/120 ¥

Adapted from Brady et al. [9]

US United States, *BP* blood pressure, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HTN* hypertension

[a] Or 120/80, whichever lower

[b] Or 130/80–139/89, whichever lower

[c] Or 140/90, whichever lower

[d] Defined by some as 20% above Grade II limit

[e] Or stage II with symptoms

Table 1 [9]. As can be seen, there are common features, including use of the 90th percentile to denote the upper limit of normal BP in children (up to 13 years in the AAP guideline, and up to 16 years in the ESH guideline), and use of the 95th percentile to denote HTN (up to 13 years in the AAP guideline, and up to 16 years in the ESH guideline). The other major similarity is adoption of static cut-points for diagnosis of HTN in adolescents [4, 5]. In the case of the AAP guideline this was done to align the pediatric guideline with the corresponding adult guideline, which was in development at the same time as the pediatric one [10].

However, as has been pointed out by others [9, 11, 12], there are notable differences as well, with the two major ones being the normative BP data used, and the second being the difference in age at which adult BP cut-points were adopted. The 2017 AAP guideline was essentially an update of the 2004 “Fourth Report” issued by the United States National High Blood Pressure Education Program [13]; that document contained normative BP data tables based on a population that contained a high percentage of children with overweight and obesity. The AAP commissioned new normative BP data tables based only on children of normal weight [4]. This change resulted in BP values that were roughly 2–3 mmHg lower for each age/sex/height group compared to those in the Fourth Report. The ESH pediatric guideline [5, 6] adopted the Fourth Report normative data, leading to differences in BP classification, most notably that the AAP guideline classifies as hypertensive youth with overweight and obesity who would be classified as non-hypertensive by the ESH criteria. The implications of this difference in classification have been discussed in detail elsewhere [12, 14]. (Both the 2004 Fourth Report and the 2017 AAP BP tables are freely available online, so are not reproduced here.)

As seen in Table 1, the AAP guideline adopts adult cut-points for adolescents starting at the age of 13 years – this was chosen because the 90th percentile BP values for teens in the new normative data tables approximated the lower adult cut-points in the AHA-ACC guideline starting at 13 years of age for both boys and girls [4, 10]. In the ESH guideline, the age of 16 years was chosen because the 95th percentile for boys of that age equaled the cut-points in the ESH adult guideline. In both guidelines, the static cut-points were applied at the same age in boys and girls. In combination with the lower cut-points in the adult and pediatric American (AAP, AHA/ACC) guidelines compared to the adult and pediatric European guidelines, following the AAP guideline will result in classification of more teens between 13 and 16 years of age as hypertensive compared to following the ESH guideline. The implications of this difference require further study, particularly correlation of a HTN diagnosis with intermediate outcomes such as left ventricular hypertrophy (LVH) and vascular changes (increased pulse wave velocity, increased carotid intimal-medial thickness), so that a definitive recommendation can be made regarding which set of guidelines is most appropriate for general use worldwide.

Blood Pressure Measurement

Although evolving technology can offer the means to measure complex functions of cardiovascular (CV) physiology, it is worth emphasizing that the basic phenotype remains BP. More importantly, all other measurements are meaningless unless BP is measured accurately. BP is a physiologic parameter which has different values along the vascular tree. It is characterized by continuous dynamic fluctuations that are the result of a complex interplay among environmental, physical, and emotional factors, all inducing BP changes [15]. Variability in BP, from a clinical viewpoint, can be seen as a source of noise that creates difficulties in assessing the individual's "true" BP level, introducing uncertainty into the assessment of a subject's BP status. This is especially true when spot clinic measurements are used [16]. These are the fundamentals for developing methods to measure BP at different points in the vascular tree, brachial or aortic root, and for establishing different protocols for office and out-of-office BP assessments [4–6].

The different approaches, beginning with how to assess BP values and following with the number and conditions of the procedure, are major issues of controversy since the results are not always in concordance. Office BP is the basis for HTN screening and diagnosis, and despite the increasing use of out-of-office BP, the former remains indispensable for defining BP phenotypes. Office BP is a widely available, permitting universal, regular screening for HTN in the pediatric age group, which is recommended from the age of 3 years old by current pediatric guidelines [4–6]. The type of the device, oscillometric or auscultatory, the number of readings per visit, the number of visits, and the reference values used to classify BP conditions are still matters of debate.

In recent years, there has been a fundamental change in the methodology for measuring office BP. As the traditional auscultatory technique requires a sphygmomanometer, a stethoscope, and a trained observer, the automated oscillometric measurement has been introduced. This measurement requires a validated device that may provide the average of a number of measurements and little training for the observer. In the auscultatory method, Korotkoff sounds are used to assess SBP (K1) and DBP (K5) while deflating the cuff [16]. Oscillometric devices measure mean BP directly from the point of maximum oscillation; neither SBP nor DBP are measured directly, but are calculated using an algorithm based on a putative relationship between the oscillations [17]. If an oscillometric method is applied, the monitor should have passed the validation procedure recommended by the British Hypertension Society [18], the American Association for the Advancement of Medical Instrumentation [19], or the ESH International Protocol [20]. Values obtained with an oscillometric device are considerably higher than those obtained by auscultatory ones. Therefore, if HTN is detected by the oscillometric method, it is recommended to be confirmed it by the auscultatory one [4–6]. Recent studies have proposed reference values using oscillometric devices [21–23]. Unfortunately, the heterogeneity of the studies, as well as differences in manufacturers' algorithms, does not permit pooling the information.

Evolving data form cohort studies based on office BP at childhood and adolescence show an incremental risk of HTN and subclinical target organ damage in early adulthood, confirming the prognostic significance of office BP measurement [24, 25]. Office BP, despite its persistence as the main reference for BP classification and HTN management, may offer limited insight into the variability and patterns of BP under living conditions. To reduce variability induced by the presence of physicians or nurses, unattended BP measurement has been introduced in the last several years, but pediatric experience is limited [26–29].

Ambulatory Blood Pressure Monitoring

The role of ambulatory BP monitoring (ABPM) over 24 h has been increasingly recognized since it offers a more representative observation of BP levels, not only allowing for a large number of measurements in a regular life environment, both day and night, during activity and while at rest, but it is also an assessment of the circadian profile [30]. ABPM is now increasingly recognized as providing useful information for the diagnosis and management of HTN and has contributed significantly to our understanding of HTN by "unmasking" BP phenomena that were not readily apparent using office BP. Values obtained using 24-h ABPM have a higher reproducibility and better relationship with the presence of organ damage than do those obtained using office BP [31–33]. Although some concerns exist about the superiority of ABPM over office BP, claiming that a mathematical artifact can explain the differences, the role of ABPM in pediatric HTN has been established, and further reinforced by the latest pediatric guidelines.

The classification of ABPM categories is based on reference values from a European study [31], which have been used as the basis of the recommended definition of HTN in the guidelines. Despite the limitations of ABPM such as observers' expertise, normative tables [34], and monitor validation, there is a consensus that the diagnosis of HTN should be confirmed by ABPM to avoid treatment for white-coat HTN. Moreover, children and adolescents with suspected masked HTN or with high-risk conditions should undergo ABPM to evaluate whether an abnormal BP phenotype exists. Repeated ABPM in research studies has established that ABPM can provide useful prognostic information. This is the case of masked HTN as a forerunner of sustained HTN [35], the risk to develop nephropathy in type 1 diabetes [36], and the relevance of strict BP control and the progression of renal failure [37]. Even in the clinical setting, repeated ABPM can be useful to determine the stability of an individual patient's BP phenotype [28].

In clinical practice, the averages of 24-h diurnal and nocturnal BP are the recommended parameters for defining HTN status and for monitoring the BP-lowering effect of antihypertensive agents. In a meta-analysis of ten long-term outcome studies performed in more than 8000 adults, BP load did not add significantly to the CV risk associated to the mean 24-h BP level [38]. The night-to-day BP ratio represents circadian variability due to the physiological nocturnal BP fall. A nocturnal BP fall of >10% of daytime values (night-day BP ratio <0.9) can be accepted as an arbitrary cut-off to define normality [15]. Abnormal circadian variability has been associated with sleep disturbance, obstructive sleep apnea, obesity, high salt intake in salt-sensitive subjects, autonomic dysfunction, chronic kidney disease, and diabetes, both type 1 and type 2 [5, 6, 32, 39]. The prognostic significance of reduced circadian variability has been claimed as a result of an association with increased organ damage. Nevertheless, resolving the circadian abnormality has not been accepted as a target [40].

The intraindividual differences between office and out-of-office BP values, white-coat and masked HTN, are the strengths of ABPM as a diagnostic tool. The reported frequency of white-coat HTN varies, perhaps as a result of the criteria used to establish the diagnosis [41]. At present, no long-term follow-up data are available for children with white-coat HTN at initial assessment, in order to know the reproducibility and the real impact of this condition. Hence, it remains to be clarified whether white-coat HTN is an innocuous phenomenon or a prelude to future sustained HTN. The inverse phenomenon, masked HTN occurs in approximately 10% of children and adolescents [42–44]. The persistence and clinical significance of the phenomenon has shown that in 40% of children, the abnormal elevation of the daytime ambulatory BP persisted over a minimum

Table 2 Recommendations for 24-h ambulatory BP measurements

During the process of diagnosis
Confirm hypertension/Detect white-coat hypertension Target organ damage (LVH, microalbuminuria) and office BP normal (masked hypertension) Type 1 and Type 2 diabetes Chronic kidney disease Renal, liver, or heart transplant Coarctation of the aorta (repaired) Severe obesity with or without sleep-disordered breathing Hypertensive response during the treadmill test Discrepancy between office BP and home BP
During antihypertensive drug treatment
Evaluate for apparent drug-resistant hypertension Assessment of BP control in children with target organ damage Symptoms of hypotension
Clinical trials
Other clinical conditions
Autonomic dysfunction
Suspicion of catecholamine-secreting tumors

Ref: Lurbe et al. [5, 6], Flynn et al. [4]

of 6 months [42]. Masked HTN has been linked to progression to HTN in youth, with the risk being higher in boys than in girls [35]. Masked HTN warrants follow-up, and if it persists, left ventricular mass should be assessed. Recommendations by scientific organizations on the use of ABPM in the process of diagnosis and evaluation treatment have been developed during the past several years and are summarized in Table 2.

Other Approaches to Blood Pressure Assessment

The role of home BP monitoring (HBPM) has been less assessed, and its use during the process of HTN diagnosis is not currently supported. Its routine use, however, is recommended by expert opinion' for the follow-up of chronic pediatric HTN subjects, when strict BP control is mandatory [4–6]. It is noteworthy that HBPM presents both lower sensitivity and positive predictive value in children and adolescents when predicting masked HTN compared to those for ABPM, although a good performance at assessing for white-coat HTN has been reported [45, 46]. This finding supports the recommendations for its use in a complementary role between regular ABPM in hypertensive pediatric patients for pharmacological treatment dose titration, assessment of BP control, and the timing of repeat ABPM. Home BP has been reported as lower than daytime ABP in children and adolescents, while the relation between home and office BP showed inconsistent patterns among studies, dependent on age, BP elevation, and office BP measurement method [47]. Several issues about the clinical application of the method, including validated devices, remain under-investigated. However, the provision of guidance regarding the proper methodology for reliable home measurement [5, 6] could help identify more children at increased CVD risk and has the potential to move the field forward [9].

Although less widely studied, the evaluation of central BP (cBP) in children and adolescents is also important because pulse pressure amplification between central and peripheral sites is greatest in youth. Mean and diastolic BP are nearly constant throughout the arterial tree, but systolic BP changes, with peripheral being higher than central or aortic BP [48]. Compared with adults, this phenomenon differs significantly in children, whose systolic BP amplification is substantial, almost twice that of adults [49]. If central systolic BP relates more closely than peripheral systolic BP to target organ damage, including LVH as some claim in adults [50], it may be very useful clinically to estimate its values in children. Nevertheless, its benefit and independence from peripheral BP remains unknown. In order to obtain an indirect assessment of hemodynamic parameters in the aortic root, noninvasive methods have been developed over the last few years. Consequently, the assessment of cBP has increased and has been introduced into many specialized clinics. The challenge of using cBP in children and adolescents is the absence of grounded reference values for classifying a given cBP as "normal" or not. In addition to the lack of reference cBP values, the high variability of the amplification phenomenon between aortic and brachial systolic BP at all ages [51] leads to a more difficult interpretation. Although more research is necessary in the future, cBP can help to identify the different phenotypes of people with isolated systolic HTN [52, 53]. In a recent study [54] the cuff-based radial tonometry and transfer function method has demonstrated its superiority over oscillometric devices in the assessment of central BP. The study also validates that the transfer function developed in adults is also valid in children.

Simultaneous assessment of peripheral and cBP has identified a condition that is called "pseudo" or "spurious HTN." O'Rourke was the first to describe this condition in six young males aged 14 to 23 years [55] characterizing it by a high peripheral systolic BP with "normal" central systolic BP and attributed to exaggerated pulse pressure amplification from central to peripheral arteries due to increased vascular elasticity. It is furthermore claimed to be probably a benign condition, although one recent study showed that almost one-quarter of youth with so-called spurious HTN developed sustained HTN on repeat ABPM after one year, and 51% evolve to white-coat HTN or normotension [56].

The potential value of central systolic BP and central pulse pressure in the assessment of adolescents with isolated systolic HTN is controversial. Central BP may be especially relevant in asymptomatic children incidentally found to have isolated peripheral systolic HTN without target organ damage [5, 6]. In children and young adults at high risk of future adverse CV outcomes (e.g., with primary HTN or chronic kidney disease), research is needed to ascertain not only which factors determine systolic amplification but also interventions that have a differential impact on central rather than peripheral systolic pressure [57]. Because of their high correlation, assessment of cBP will only offer incremental value over peripheral BP measurement if cBP proves superior at identifying target organ damage.

Blood Pressure During Exercise

Assessing BP during exercise has an established role in the evaluation of HTN and CV prognosis in adults, but fewer data examining its role are available in children and adolescents. BP measurements taken during exercise testing provide valuable information on a potentially inadequate hemodynamic response to acute exercise.

Calculated heart rate and systolic BP during exercise are positively associated with future resting BP in longitudinal studies [58, 59]. Likewise, an exaggerated systolic BP during exercise has also been reported in children at risk for early damage in vascular structure and functioning [60, 61] while an exaggerated BP response to treadmill exercise has been observed in children with white-coat HTN [62]. Despite these association studies, the clinical usefulness of BP response to exercise testing in children and adolescents is limited by the absence of reference values that include children and adolescents, which only exist for those 12–17 years old [63]. The ESH Guideline for HTN in adults suggest performing 24-h ABPM in subjects with a hypertensive response during the treadmill test searching for the possible presence of masked HTN [8]. Even though evidence in youth is lacking, this recommendation can be considered in adolescents.

Diagnostic Evaluation

The potential causes of HTN in childhood are broad and discussed at length in ▶ Chap. 62, "Pathophysiology and Epidemiology of Hypertension in Children." This requires a careful history and examination. This includes an awareness of syndromes associated with HTN due to renal, cardiac, endocrine, or other causes. Both recent pediatric guidelines [4–6] have detailed discussions of differential diagnosis and characteristic physical exam findings, which the interested reader should consult for additional guidance.

The changing epidemiology of childhood HTN has meant a revision in the suggested diagnostic evaluation. Both the AAP and ESH guidelines recommend a focused first-line screen in the absence of signs or features to suggest secondary disease, which would include age ≤5 years, stage 2 HTN, or a failure to adequately control BP with a single agent. Common components of the AAP and ESH guidelines are: urinary tract ultrasound, urinalysis, blood urea, serum electrolytes, and creatinine. A lipid screen and echocardiography for end organ damage are also recommended. Other tests, including plasma renin and aldosterone, cortisol, metanephrines, and catecholamines, all fall under the category of second-line tests to be performed in selected cases only.

This change reflects the importance of interpreting results within the clinical context they are performed. Even if test performance is consistent across subgroups, which is often not the case, unless the test is very accurate then a low pretest probability will equate to a low post-test probability. Considering as an example the use of free plasma metanephrines to detect pheochromocytoma, which has a sensitivity and specificity of approximately 95% [64]: If the pretest probability is 1%, then the post-test probability following a positive result will be 16%. Otherwise put, in this setting 84% of all positive results will be a false positive.

Evaluation for Secondary Causes

It is important to first rule out common presentations that do not require extensive evaluation, such as incorrect BP measurement of BP or white-coat HTN. Pain is frequently the cause of HTN in an inpatient setting, and treatment is best directed toward this rather the secondary increases in BP. Similarly, if volume overload is present then this need be identified and treated. After confirming that the child does have HTN requiring further investigation, it can be useful to consider potential causes by system.

Renal and Monogenic Causes of Hypertension

Renal parenchymal or vascular disease is the most common secondary cause of childhood HTN [65–67]. ▶ Chap. 64, “Renovascular Hypertension in Children: Evaluation and Management” details the evaluation of renovascular disease, so we will not review that here.

In most instances, a normal ultrasound, serum creatinine, and urinalysis are sufficient to rule out renal parenchymal abnormalities. While a negative or trace result on dipstick urinalysis has a high negative predictive value, if there is a strong index of suspicion or the urine is very dilute then a urine protein-to-creatinine ratio is appropriate [68]. An abnormal urine sediment may suggest glomerulonephritis, resulting in HTN due to activation of the renin-angiotensin-aldosterone system, sympathetic activation, and/or volume overload [69, 70]. Proteinuria and hematuria may also occur in the setting of thrombotic microangiopathy secondary to severe HTN, in association with hemolytic anemia, thrombocytopenia, and an elevated serum creatinine [71]. Less often the casual relationship is reversed, and atypical hemolytic uremic syndrome presents with HTN [72].

A dimercaptosuccinic acid (DMSA) scan may detect small renal scars not present on ultrasound, with abnormalities detected in 5–10% of children with a history of HTN but no history of urinary tract infection (UTI), including where other imaging modalities are unremarkable [73]. Whether these scars are the cause of HTN in the majority of these cases is unclear. While small parenchymal defects are common following childhood UTI, they usually do not result in clinical sequelae [74]. Hence, most centers will reserve DMSA scans for children with a history of recurrent febrile UTI or well-documented pyelonephritis.

An elevated plasma renin activity (PRA) or serum aldosterone may also point toward renal disease, or rarely a renin secreting tumor [75]. However, renal causes of HTN are usually detected on imaging and are not necessarily excluded by a normal PRA. Furthermore, an elevated PRA does not necessarily differentiate primary from secondary HTN [65, 76].

Low PRA values are important to identify because of an association with monogenic forms of HTN. These disorders mostly involve renal salt handling and steroid metabolism. Directed therapies exist, including mineralocorticoid receptor antagonists, epithelial sodium channel (ENaC) blockers, and glucocorticoids. Of note, these are not standard first-line agents and may not be used at all if the underlying diagnosis is missed. Beyond the evaluation of PRA, which based on the data available thus far appears to be reduced in most

Table 3 Monogenic disorders causing hypertension

	Inheritance	Age	K	Renin	Aldo	Urine steroid profile	Therapy
AME	AR	I, C, A	↓ or N	↓	↓	AbN	Spironolactone, eplerenone
GRA	AD	I, C	↓ or N	↓	↑ or N	AbN	Amiloride, triamterene, glucocorticoids
CAH	AR	I	↓ or N	↓	↓	AbN	Spironolactone, eplerenone
LS	AD	C, A	↓ or N	↓	↓	N	Amiloride, triamterene
GS	AD	C, A	↑ or N	↓	↑ or N	N	Thiazide

Modified from Ferguson and Flynn [195]

AME apparent mineralocorticoid excess, *GRA* glucocorticoid remedial aldosteronism, *CAH* congenital adrenal hyperplasia, *LS* Liddle syndrome, *GS* Gordon syndrome, *AR* autosomal recessive, *AD* autosomal dominant, *I* infancy, *C* childhood, *A* adulthood, *N* normal, *AbN* abnormal, ↓ decreased, ↑ increased

cases of monogenic HTN, the known disorders can be categorized based on the serum aldosterone level and other electrolytes (Table 3) [77–80].

A raised aldosterone level, low PRA, family history of HTN early in life and cerebrovascular accidents suggests glucocorticoid-remediable aldosteronism (GRA) [80].[15] GRA is inherited in an autosomal dominant fashion and was one of the first monogenic causes of HTN identified. The specific defect is a fusion of the CYP11B1 and CYP11B2 genes, resulting in an aldosterone synthase product that is responsive to adrenocorticotropic hormone (ACTH), and thus aldosterone levels are elevated and related to ACTH levels rather than PRA [81]. Overproduction of cortisol C-18 products is detectable in the urine and can establish the diagnosis in the absence of genetic testing. While GRA is a rare condition in the population overall, polymorphisms in the CYP11B1 and CYP11B2 are reported to have a digenic relationship to HTN [82].

Hyperkalemia with a low PRA, hyperchloremic acidosis, and normal or low aldosterone level suggests pseudohypoaldosteronism type II or Gordon syndrome [78]. Four underlying genes have been implicated, all resulting in increased sodium, potassium, and chloride resorption in the distal convoluted tubule [83, 84]. Mutations of cullin-3 (CUL3) tend to have a more severe phenotype and present in childhood, consistent with CUL3 being expressed throughout the nephron, including proximal convoluted tubule [83]. The other identified mutations, KLHL3 (recessive and dominant forms), WNK4, and WNK1, may present at any age, although the majority of causes are not identified until adulthood. Thiazide diuretics reverse the electrolyte abnormalities and lower BP in affected patients [83].

The presence of hypokalemia in conjunction with low PRA points to congenital adrenal hyperplasia (CAH), apparent mineralocorticoid excess (AME), or Liddle syndrome. In contrast to the most common form of CAH, 21-hydroxylase deficiency, which presents with salt wasting and hyperkalemia, 11β-hydroxylase and 17α-hydroxylase defects result in an accumulation of intermediate steroids with mineralocorticoid activity. Aldosterone is reduced, but deoxy-corticosterone (DOC) accumulates, activating the mineralocorticoid receptor, leading to sodium retention, HTN, and hypokalemia [85]. 11β-hydroxylase is associated with virilization or pseudoprecocious puberty, whereas 17α-hydroxylase is associated with under-virilization or female external genitalia in XY individuals [86]. In AME, a HSD11B2 gene mutation results in impaired 11β-hydroxysteroid dehydrogenase conversion of cortisol to cortisone within the kidney, the former and not the latter being capable of activating the mineralocorticoid receptor [87]. The urinary steroid profile will be abnormal in both CAH and AME, including an excess of the aforementioned metabolites [79, 88]. Licorice contains glycyrrhetinic acid, which inhibits 1β-hydroxysteroid dehydrogenase with high levels of consumption replicating AME [89, 90]. One final condition presenting with a low PRA and aldosterone is Liddle syndrome, caused by an upregulation of the epithelial sodium channel (ENaC) [91]. Consistent with the underlying mechanism, Liddle syndrome presents with hypokalemia, HTN, and alkalosis [77].

Autosomal dominant HTN with brachydactyly type E (HTNB) is a well-described syndrome caused by mutations of PDE3A encoding phosphodiesterase 3A [92, 93]. Different to the other causes of monogenic HTN discussed, PRA is normal and parathyroid hormone related peptide (PTHrP) is dysregulated [86].

Monogenic disorders of HTN are rare, both in the general population and pediatric HTN clinics where screening has been performed. Regardless, within increasing availability of genetic testing it may be that more children with monogenic disorders are identified and phenotypes further delineated.

Cardiac Causes of Hypertension

Coarctation of the aorta may present with HTN, and while many cases are detected antenatally, it is common that the diagnosis is not apparent until after birth [94]. Children may present with reduced femoral pulses and lower limb BP, but in children who do not have clinical signs of lower limb hypoperfusion the difference between upper and lower limb BP is not an accurate predictor of coarctation [95]. It is now recognized that HTN occurs frequently after coarctation repair, and is associated with CV events later in life [96–98]. Importantly, HTN among these children is often masked and thus regular ABPM recommended [4–6, 32, 39, 99].

Pheochromocytoma and Paraganglioma

Pheochromocytoma and paraganglioma (PPGL) are endocrine neoplasms that cause HTN by way of catecholamine production and release. Both are derived from neuroectoderm cells of the neural crest, but differ by location and propensity to catecholamine secretion [100]. Pheochromocytoma arise from chromaffin cells of the adrenal medulla and most secrete one or more catecholamines. Paraganglioma arise from autonomic ganglia, the paravertebral sympathetic ganglia, or the parasympathetic ganglia of the glossopharyngeal and vagal nerves within the neck and base of skull. Paraganglioma of the sympathetic ganglia are more likely to secrete catecholamines than those of parasympathetic ganglia, which may be relatively silent [101, 102]. PPGL are typically well-differentiated tumors, but a minority of cases develop distant metastases [103]. Pheochromocytoma are more common than paraganglioma [104, 105].

Given the diversity of tumor biology and overlap between normal and affected individuals for singular biomarkers, the use of multiple tests is required, usually including fractionated metanephrines (normetanephrine and metanephrine) and catecholamines (norepinephrine, epinephrine, and dopamine). Metanephrines are increasingly preferred for the detection of pheochromocytoma, as they are more stable analytes than catecholamines and are directly produced within pheochromocytomas by catechol-O-methyltransferase (COMT) [106]. Dopamine or its metabolites 3-methoxytyramine and homovanillic acid (HVA) may also need to be measured, as some paragangliomas secrete dopamine only [107]. The most accurate test is free plasma metanephrines after 30 min in the supine position [108, 109]. Seated testing may be used to rule out a PPGL, but because values among normal individuals are raised in the seated position by more than those with already elevated levels due to PPGL, it becomes more difficult to separate affected from nonaffected individuals [110, 111].

The largest prospective, diagnostic test accuracy study for PPGL found the sensitivity and specificity of free plasma metanephrines to be 99% (95%CI 96 to 100%) and 89% (95%CI 87 to 92%), respectively [109]. Fractionated urine metanephrines were nearly as sensitive (97%, 95%CI 92 to 99%), but not as specific (69%, 95%CI 64 to 72%). The accuracy of fractionated urine metanephrines may be close to that of plasma metanephrines in populations where the disease prevalence is high [108]. Urine total metanephrines and vanillylmandelic acid (VMA) are highly specific but not sensitive tests for PPGL [109]. The upper limit of normal for free plasma metanephrines is age dependent, with normetanephrine levels substantially higher among infants compared to older children [112].

Whereas mildly elevated catecholamine and metanephrines are common, a result greater than

3 to 4× the upper limit of normal, or elevation in both metanephrine and normetanephrine make a false positive less likely [109, 113]. Longitudinal trends may be useful in cases where there is an elevation less than 2× the upper limit of normal, increasing values potentially indicating PPGL [114, 115].

Medications are an important cause of false positive and negative results. Any drug that impacts catecholamine handling directly or indirectly can interfere with testing, including many agents used in the management of PPGL. These are summarized in Table 4. Increased sympathetic activity due to exercise or illness may also result in false positive results [110, 122, 123]. Diets rich in catecholamines may cause elevations in plasma and urine levels of 3-methoxytyramine, and fractionated urine metanephrines also [124].

When PPGL is suspected, CT with contrast is recommended as the initial imaging modality [105]. The abdomen and pelvis are usually imaged first, given this is where most tumors are located. MRI has a similar sensitivity and specificity to CT, and is also useful for detecting base of skull and neck paragangliomas [125]. Nuclear medicine studies are required in the presence of an SDHB mutation, pheochromocytoma >5 cm, or paraganglioma [126]. 123-MIBG is commonly used, but its sensitivity is less in SDHB associated tumors and paragangliomas [127]. In these instances ^{18}F-fluorodopamine (^{18}F-FDA), ^{18}F-fluorodihydroxyphenylalanine (^{18}F-FDOPA), and ^{18}F- fluoro-2-deoxy-D-glucose (^{18}F-FDG) are alternatives [128].[7] As with biochemical testing for PPGL, many drugs including commonly used antihypertensive agents interfere with testing, including alpha and beta blockers, and calcium channel blockers [129, 130]. Since up to 80% of children with PPGL will have an underlying mutation identified [105, 131], genetic testing should be performed concurrently with the biochemical and imaging workup.

Endocrine Disorders

Many endocrine disorders may cause HTN. Cushing syndrome is one differential diagnosis, commonly considered in the obese child with HTN. Key differences seen in Cushing syndrome compared to most HTN in obesity are a loss of linear growth and pubertal delay. Other features of glucocorticoid excess may be present such as a round face, increased fat at the base of the neck, striae, bruising, and proximal myopathy. Recommended first-line investigations for suspected Cushing syndrome include the low-dose dexamethasone suppression test, 24-h free urinary cortisol, and late-night salivary cortisol [132–134]. Either cortisol test should be performed twice to minimize the impact of day-to-day variation.

Table 4 Drugs that may impact metanephrine or catecholamine levels

	Plasma metanephrines	Plasma catecholamines	Urine metanephrines	Urine catecholamines
Beta-blockers	↑ M	°Δ	↑ NM/M	↑ NE/E
CCB	°Δ	↑ NE	°Δ	↑ NE/E
Phenoxybenzamine	↑ NM	↑ NE	↑ NM	–
Selective α-blockers	°Δ	°Δ	°Δ	↑ NE
Clonidine	↓ NM	↓ NE/E	–	–
Stimulants	↑ NM/M	–	↑ NM/M	–
Acetaminophen[a]	↑ NM	°Δ	↑ NM/M	↑ E

Selective serotonin reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors, and levodopa may also impact metanephrine and catecholamine levels

References: Eisenhofer et al. [116], Lenders et al. [117], Karas et al. [118], Bouhanick et al. [119], Lenders et al. [120], Davidson [121]

CCB calcium channel blockers, *NM* normetanephrine, *M* metanephrine, *NE* norepinephrine, *E* epinephrine, *D* dopamine, °Δ no change, – no published data

[a] Dependent on the electrochemical detection method used, also relevant to sulfasalazine

Children with signs or symptoms of thyroid disease should have a thyroid stimulating hormone (TSH) level checked as a screen. Many obese children have slight elevations of TSH, yet many children with abnormal thyroid function tests do not have HTN [135, 136]. Elevations of TSH in many of these children will be normal on repeat testing, and/or will normalize with weight loss.

Several other important endocrine diagnoses can present with HTN. Adrenal adenomas are rare in children, but can occur in the context of specific calcium (CACNA1D) and potassium channel mutations (KCNJ5), described with neuromuscular abnormalities and a strong family history of HTN, respectively [137, 138]. While growth hormone excess (gigantism or acromegaly) is overall a rare condition, it is commonly associated with HTN and should be considered in the presence of coarse facial features, rapid height gain, and acral enlargement [139, 140]. HTN can be seen with hypercalcemia due to primary hyperparathyroidism, hypervitaminosis D, and in the setting of cancer with or without direct bone involvement [141, 142].

Other Causes of Secondary Hypertension in Children

Among infants, chronic lung disease appears to be a common cause of secondary HTN. Many of these children have other risk factors, including prematurity, umbilical artery catherization, and previous acute kidney injury [143, 144]. With improving outcomes among extremely premature babies the importance of preventing future CV events is likely to grow [145].

Intraventricular hemorrhage in the neonatal period is also associated with HTN [146]. In older children other intracranial events, including raised intracranial pressure and seizures, can produce HTN by altering autonomic output. Hence, in children with neurologic findings, brain imaging is required to rule out a space occupying lesion. Similarly, an electroencephalogram may be indicated where there is concern about undetected seizure activity. Peripheral autonomic dysfunction, for example with Guillain-Barre syndrome, may cause HTN also [147].

Several causes of HTN among children with cancer have been discussed, including fluid overload, PPGL, hypercalcemia, and kidney disease. In addition to PPGL, other tumors such as neuroblastoma may secrete catecholamines. As for PPGL, when testing for neuroblastoma, plasma 3-methoxytyramine may be a better measure of dopamine production, and normetanephrine of other catecholamine excess; elevated plasma 3-methoxytyramine or normetanephrine having a sensitivity of 98% compared to 90% for elevated urine dopamine, VMA, or HVA for neuroblastoma ([148], Pietzsch et al. 2020). Renin-secreting tumors are a rare but well described cause of HTN. These include some Wilms and renal cell carcinomas, or juxtaglomerular cells tumors, which may require cross sectional imaging for detection [149]. Extrinsic compression of the renal artery by abdominal masses and renal vein thromboses are not uncommon in the setting of childhood cancer.

Heavy metals, for example with lead or mercury, may cause HTN and be considered for testing where there is a high community prevalence or clinical suspicion of toxicity [150, 151].

End-Organ Damage and Stratifying the Risk of Downstream Sequelae

The ultimate goal of HTN management is to prevent progression to downstream events. While data linking HTN in early childhood to CV events are limited, studies across multiple populations have observed an association between HTN in adolescence and clinical outcomes, including mortality, CV events, stroke, and end-stage kidney disease [152–154]. With this in mind, it is important to evaluate other components of cardiometabolic and kidney risk simultaneously with BP.

Metabolic syndrome (MetS), the concurrence of dyslipidemia, impaired glucose tolerance, central obesity, and HTN, confers a substantial increase in long-term cardiovascular risk [155]. Elevated childhood BP has been linked to development of MetS in adulthood in longitudinal cohort studies [156], underscoring the long-term implications of high childhood BP. From a life-course perspective, while individuals with such clusters of risk factors are primed to develop disease due to genetic predisposition and early life exposures, trajectories can be altered by intervention prior to the development of established end-organ disease [157].

At the time of evaluating a child or adolescent with HTN, however, interest is usually focused on identifying more proximate effects of HTN such as LVH. Echocardiography is recommended for assessment of LVH in current pediatric guidelines given that electrocardiography has a low sensitivity for detection of LVH in childhood [158]. LVH is common among children with HTN, being present in 15–30%, and is correlated with systolic BP load [159, 160]. There are some differences between guidelines, but the indexation of left ventricular mass index (LVMI) to an exponent of height is standard, as this value is not age dependent [161]. Values of 38.6–51 $g/m^{2.7}$ have been proposed to define LVH, a level associated with an increased risk of CV events among young adults and greater than the 95th centile among healthy children [162, 163]. When present, serial measurements are useful to guide treatment response. LVMI is correlated with BP improvements while taking antihypertensive therapy, with a reduction in LVMI after commencing antihypertensive therapy best described among children receiving angiotensin converting enzyme inhibitors (ACEI) [164–166].

Although research studies have identified a link between high childhood BP and narrower retinal vessels [167], it is likely that development of clinically detectable hypertensive retinopathy is relatively uncommon among children [168]. Therefore, routine referral for formal ophthalmology review in most children and adolescents with HTN is not required. However, retinal abnormalities are more common among children presenting with hypertensive crises, and these may result in permanent loss of vision [169]. Hence, ophthalmology review is still recommended for children presenting with severe HTN or visual symptoms.

The use of carotid intima-media thickness (cIMT) and pulse wave velocity (PWV) to measure vascular structure and function is appealing as a method of determining intermediate vascular phenotypes on the pathway to CV disease in later life.

[170]. Both CIMT and PWV correlate with outcomes among adults, including CV events and chronic kidney disease (CKD) progression [171, 172]. As for LVMI, there remains debate about the clinical impact of modest improvements in risk prediction for these tests above that of routinely measured biomarkers [173]. These and other vascular markers, such as flow-mediated dilatation and augmentation index, remain primarily research tools for the time being. Albuminuria also currently falls into this category. Although it is an attractive biomarker and an independent predictor of risk in other populations, both as a measure of kidney disease and vascular dysfunction [68], data among hypertensive children are limited, and the clinical utility of any additional quantification is unclear [174].

Therapy of Hypertension

Non-pharmacologic

Comprehensive lifestyle changes addressed at reducing factors responsible for high BP are recommended as initial actions to control high BP in children and adolescents [4–6]. Considerable advances have been made in recent years in identifying the main condition associated with and considered responsible for high BP in children and adolescents, overweight and obesity [1]. More limited evidence, albeit limited, has been compiled on the results of corrective interventions. Therefore, at present the most recommended approach to the treatment, of obesity-associated HTN consists of weight reduction through achieving a healthy lifestyle, characterized by eating healthy, performing physical activity, avoiding sedentary behaviors, and obtaining good quality sleep. Currently this treatment's approaches lead to positive outcomes only in patients where there is an adherence to intervention that is maintained over time.

Unfortunately, these recommendations are often difficult to achieve, especially for adolescents. In order to achieve these objectives among this population, the goals need to be established realistically, tailored to individual and family characteristics, involving the family as partners in the behavioral change process, providing educational support and materials [4–6]. Motivational interviewing approaches have been shown to be effective in achieving weight loss in the clinical trial setting [175, 176] and should be considered when treating children and adolescents with overweight and obesity.

There is no doubt about the health benefits associated with regular physical activity in children and adolescents. It has been demonstrated that regular physical activity has cardiovascular benefits. In fact, in a recent meta-analysis conducted in adults it has been established that physical activity reduced BP among adults with normal BP, prehypertension, and HTN [177]. In pre-pubertal obese children regular physical activity training for 60 min 3 times/week over 3 months resulted in a significant reduction of BP, not only when measured in the office, but also when monitored for 24-h BP. Higher levels of physical activity are associated with lower BP and results suggested that the volume of activity might be more important than the intensity [178]. The World Health Organization recommends at least 60 min daily of moderate- to vigorous-intensity physical activity [179]. Recent Guidelines on Physical Activity published by the US Department of Health and Human Services are consistent with the World Health Organization, and emphasized vigorous-intensity physical activity at least 3 days a week. Although children under 5 years of age benefit from being active, more research is needed to determine what dose of physical activity provides the greatest health benefits [180].

To better plan physical exercise, an evaluation of cardiorespiratory fitness may provide useful information. Physical fitness represents an integrated measure of all the functions and structures, musculoskeletal, cardiorespiratory, hematocirculatory, endocrinometabolic, and psychoneurological functions, involved in the practice of physical activity or exercise. In children and adolescents, despite the scarce number of studies, strong associations with cardiometabolic abnormalities have been described [181, 182] and should be taken into consideration for developing effective and personalized interventions. Strong evidence indicates that performing moderate-to-vigorous physical activity, particularly aerobic exercise, and improving cardiorespiratory fitness reduce BP levels and the incidence of HTN. Consequently, to lower the risk of HTN, promoting physical activity and improving fitness, especially cardiorespiratory fitness, should be encouraged [183].

Prospective studies have shown the effects of diet on lifetime BP. Consumption of >1 servings of dairy products/day and >2 servings of fruits and vegetables/day throughout adolescence has shown to lead to about a 35% lower risk of elevated BP [184]. However, children's vegetable consumption is generally below recommended levels. Therefore, future research should investigate ways to encourage vegetable intake at times when vegetables are not regularly eaten, such as for breakfast and as snacks, while considering which other, potentially competing, foods are presented alongside vegetables.

There are other considerations that need to be taken into account for overall prevention of HTN. For example, a reduced saturated fat–diet from infancy decreases BP [185] and the association of higher sugar sweetened beverage consumption with higher systolic BP in adolescents [186]. High salt intake in children and adolescents is positively correlated with high systolic BP and an elevated risk of HTN [187] and a meta-analysis has shown that a 3 g/day reduction of salt intake leads to a decrease in SBP and DBP of 1.2/1.3 mmHg [188]. Starting in newborns, a 15-year follow-up study has suggested a predictive role of the effect of salt intake in early life on BP [189]. Excessive salt intake seems to be not only linked with BP elevation but also to other cardiovascular risk factors since salt produces a reduction in vascular nitric oxide bioavailability that limits endothelium-dependent dilation [190].

In addition to the dietary factors outlined above, there are other non-pharmacologic measures that merit consideration. The relevance of tobacco smoking has been widely demonstrated. Even after correction for other risk factors, such as body mass index, parental HTN, or birth weight, parental smoking independently affected systolic BP in preschool children [191]. Implementing a smoke-free environment at home and in public places may provide a long-term cardiovascular benefit even to young children, and may even help to prevent the uptake of cigarettes in adolescents. Yoga and other stress reduction techniques have shown promise in reducing BP in some studies [192, 193].

Lifestyle interventions are important because they can delay the need for drug treatment or complement the BP lowering effect of antihypertensive treatment. Application of the Dietary Approaches to Stopping Hypertension diet in adolescents with high BP reduced BP and improved endothelial function [194]. Aside from the condition's associated with overweight and obesity, non-pharmacological treatment should be considered as an accompanying measure to drug treatment in secondary form of HTN. Moreover, lifestyle interventions such as regular exercise, healthy eating, weight control, sodium restriction, and smoking cessation all have health benefits beyond their impact on BP.

Pharmacologic Therapy

Lifestyle measures do not result in immediate reduction of BP, and may not reduce BP sufficiently in children and adolescents with HTN at or above stage 2. Additionally, there may be other considerations such as the presence of underlying conditions, symptoms, or hypertensive target-organ damage, which may justify starting an antihypertensive medication at the time of or shortly after diagnosis. Indications from current pediatric HTN guidelines [4–6] for initiating antihypertensive medications are summarized in Table 5.

Choice of initial medication is often confusing. While some experts recommend using a pathophysiologic approach to the use of antihypertensive medications in children and adolescents, in general the choice of agent is left up to the individual prescriber. Consideration should be given to special conditions such as underlying CKD or diabetes, or the concurrent presence of migraine headache when choosing a medication, similar to the "compelling indications" approach recommended in the past for adults. Some medications may be contraindicated in special circumstances, such as angiotensin converting enzyme inhibitors (ACEI) in patients with bilateral renal artery stenosis [5, 6]. Additionally, if the child or adolescent is found to have a monogenic form of HTN there is often a specific agent that will target the mechanism of the HTN. These and other considerations have been discussed in detail elsewhere [195].

Table 5 Indications for antihypertensive drug treatment

Primary Hypertension
Failure to respond to lifestyle modification Stage 2 hypertension without an easily modifiable contributing factor Symptomatic hypertension
Underlying high-risk conditions
Chronic kidney disease Type 1 and type 2 diabetes Known hypertensive target-organ damage Repaired coarctation of the aorta Other secondary forms of hypertension
Monogenic Hypertension

Ref: Flynn et al [4], Lurbe et al [5].

First-line antihypertensive agents recommended by the AAP include long-acting calcium channel blockers, ACEI, angiotensin receptor blockers (ARB), and thiazide diuretics [4]. The ESH guideline cautions against use of thiazide diuretics as initial therapy because of their potential adverse effects on glucose metabolism [5, 6]. Other classes of agents including beta- and alpha-adrenergic blockers are generally reserved for second- or third-line use given their known side effects. Monotherapy with long-acting drugs that can be dosed once daily is preferred, but additional agents may be needed to achieve optimal BP levels (see below).

Historically, drugs prescribed for children were not studied rigorously to assess for efficacy, safety, or appropriate dosing regimens. The passage of the Federal Drug Administration (FDA) Modernization Act in 1997 heralded an era of increased rigor with respect to pediatric drug development by providing financial incentives to industry sponsors for conducting pharmaceutical trials in children [196]. Subsequent legislative efforts, including new pediatric drug regulations in Europe [197], have facilitated ongoing attention to this area. Antihypertensive drugs represent one of the largest groups of medications impacted by these efforts [198]. Consequently, a growing number of antihypertensive medications from different classes have been studied in children and adolescents, resulting in more of these agents receiving FDA-approved labeling for use in children and adolescents [199]. Many of the antihypertensive medication-related recommendations in the AAP and ESH guidelines are based on the information derived from those trials.

Antihypertensive drugs in children and adolescents are generally prescribed in a stepped-care manner: The child is initially started on the lowest recommended dose, then the dose is increased until the highest recommended dose is reached, or until the child experiences adverse effects from the medication, at which point a second drug from a different class should be added, and so on, until the desired goal BP is reached. Pediatric dosing recommendations for selected antihypertensive medications can be found in Table 6.

Recommended treatment targets for children and adolescents with HTN have evolved over time as new data have become available on optimal BP levels. For example, the AAP guideline adopted a lower general treatment goal, office BP below the 90th percentile, for all children and adolescents than in prior US guidelines largely because of new adult data demonstrating that lower BP has a beneficial effect on long-term outcomes [4]. The ESH pediatric guideline maintained a general office BP goal of the 95th percentile for children and adolescents with primary HTN, but the same goal for children and

Table 6 Selected medications for oral therapy of childhood hypertension[a]

Class	Drug	Dosing[b]
Alpha adrenergic blockers	Prazosin Doxazosin	0.05–0.1 mg/kg/day divided TID; Max: 0.5 mg/kg/day 0.5–1.0 mg/day; Max 16 mg/day
Angiotensin receptor blockers	Candesartan Losartan[a] Olmesartan Valsartan	Start: 0.2 mg/kg/day up to 8 mg/day; Max: 0.4 mg/kg/day up to 32 mg/day (32 mg) Start: 0.7 mg/kg/day divided BID; Max: 1.4 mg/kg/day divided BID; (100 mg) Start: 10–20 mg/day; Max: 20–40 mg/day (40 mg) Start: 1.3 mg/kg/day up to 40 mg/day; Max: 2.7 mg/kg/day up to 160 mg/day (320 mg)
Angiotensin converting enzyme inhibitors	Enalapril Lisinopril[c] Ramipril	Start: 0.08 mg/kg/day divided BID; Max: 0.6 mg/kg/day divided BID (40 mg) Start: 0.07 mg/kg/day; Max: 0.6 mg/kg/day (40 mg) 1.25–2.5 mg/day; Max: 20 mg/day (ESCAPE trial used 6 mg/m^2/day)
Beta-adrenergic blockers	Atenolol Propranolol[c] Metoprolol Succinate	Start: 0.5 mg/kg/day divided BID; Max: 2 mg/kg/day divided BID (100 mg) Start: 1 mg/kg/day divided BID; Max: 8 mg/kg/day (640 mg) Start: 1 mg/kg/day up to 50 mg; Max: 2 mg/kg/day up to 200 mg (200 mg)
Calcium channel blockers	Amlodipine[c] Extended-release nifedipine	Start: 0.1 mg/kg/day; Max: 0.6 mg/kg/day (10 mg) Start: 0.2 mg/kg/day; Max: 3 mg/kg/day (120 mg)
Mineralocorticoid Receptor antagonists	Eplerenone Spironolactone	25 mg/day; Max 100 mg/day 1 mg/kg/day; Max: 3.3 mg/kg/day (100 mg)
Thiazide diuretics	Hydrochlorothiazide Chlorthalidone	Start: 1 mg/kg/day; Max: 2 mg/kg/day (50 mg) Start: 0.3 mg/kg/day; Max: 2 mg/kg/day (50 mg)

BID twice-daily, *d* day, *kg* kilogram, *mg* milligram

[a] Starting and maximal doses given either as absolute dose or as weight-based ranges. Maximum adult dose in parentheses. Always consult updated pediatric prescribing information

[b] All drugs can be administered once-daily except as otherwise indicated

[c] Suspension preparations commercially available

adolescents with secondary HTN as in the AAP guideline [5, 6]. Both organizations recommend using an ABPM-based goal for children with CKD based on data from the ESCAPE trial showing that that strict BP control, defined as 24-h mean arterial pressure (MAP) below the 50th percentile leads to improved long-term renal survival [37].

Once medication treatment has been initiated, it is important to ensure that BP goals are reached and maintained. Children and adolescents treated with lifestyle measures only should be seen every 3–6 months to monitor the effect of treatment and to encourage continued adherence. Those started on antihypertensive medications should be seen frequently at first, perhaps every 6–8 weeks, so that drugs can be titrated, and then less often once a stable regimen and the goal BP attained. HBPM is a useful tool to help determine response to medication treatment. Repeat ABPM may also be used, and is mandatory in children and adolescents with HTN, specifically those with CKD to monitor the 24-h MAP and ensure that the targets based on data from the ESCAPE trial are being met [4–6]. Finally, children treated with specific medication classes – ACEI, ARBs and diuretics – require periodic laboratory monitoring to assess for electrolyte disturbances and other medication-related toxicities, and those with known target-organ effects such as LVH should undergo periodic reassessments as indicated.

Acute Severe Hypertension

Acute severe HTN, defined as significant BP elevation with or without evidence of acute hypertensive target organ damage, requires prompt therapy to prevent and/or ameliorate further target organ damage. Traditionally the terms hypertensive urgency and hypertensive emergency have been used to describe this condition, but since the distinction between these is arbitrary, we prefer the term “acute severe HTN.”

The most common causes of acute severe HTN in children and adolescents are summarized in Table 7. While some patients may have no identifiable cause, the majority of affected patients have secondary causes of HTN [200]. Mechanisms of acute severe HTN may include volume overload with or without renal dysfunction, and/or activation of the renin-angiotensin-aldosterone system. Etiology and pathophysiology have been discussed in more detail elsewhere [201, 202]. While a variety of organs may be affected in patients with acute severe HTN, the central nervous system is the most commonly affected target organ.

Treatment of acute severe HTN requires continuous monitoring of BP and meticulous patient monitoring to prevent excessively rapid lowering of BP. In patients with the most significant target organ involvement, BP should be lowered no more than 25% of the planned reduction over the first 8 h to prevent compromise of organ perfusion (e.g., cerebral, coronary, or renal ischemia). The ultimate BP target in the acute phase is usually the 95th percentile, and can be achieved over the next 24–48 h [201, 202]. In most patients, this will require administration of intravenous antihypertensive medication(s), most commonly labetalol or nicardipine. In less severely affected patients, oral medications may be considered. Drug dosing for patients with acute severe HTN has been described in detail elsewhere [203]; suggested drug doses are presented in Table 8.

Pheochromocytoma and Paraganglioma

Many children with pheochromocytomas initially present acutely with severe, symptomatic HTN of unknown etiology [105]. Such children require careful immediate stabilization and gradual BP reduction as described in below until the diagnosis is made. Although theoretically the α- and β-adrenergic blocker labetalol may seem uniquely well-suited for treatment of HTN caused by a pheochromocytoma, in practice a continuous infusion of any of the intravenous antihypertensives listed in Table 8 could be utilized for initial BP management. Phentolamine has also been advocated in such patients, although pediatric experience with this agent is limited.

Once the diagnosis of pheochromocytoma has been made, the child’s BP should be stabilized

Table 7 Causes of acute severe hypertension in children and adolescents

Kidney Disease	**Malignancies**
Acute glomerulonephritis Reflux nephropathy Acute kidney injury Polycystic kidney disease Hemolytic-uremic syndrome End-stage kidney disease at presentation	Pheochromocytoma Wilms tumor Neuroblastoma
Vascular	**Other**
Aortic coarctation (thoracic, abdominal) Renal artery stenosis Vasculitis (Takayasu's, Polyarteritis nodosa, others)	Medication noncompliance in a patient with known hypertension Drug-induced, especially abrupt withdrawal of clonidine or beta-blockers Primary hypertension (unusual)

Table 8 Recommended antihypertensive medications for acute severe hypertension

Useful for severely hypertensive patients with life-threatening symptoms				
Drug	**Class**	**Dose**	**Route**	**Comments**
Esmolol	β-adrenergic blocker	100–500 mcg/kg/min	iv infusion	Very short-acting—constant infusion preferred. May cause profound bradycardia
Hydralazine	Direct vasodilator	0-1-0.2 mg/kg/dose up to 0.6 mg/kg/dose	iv, im	Should be given q 4 h when given iv bolus
Labetalol	α- and β-adrenergic blocker	bolus: 0.20–1.0 mg/kg/dose, up to 40 mg/dose infusion: 0.25–3.0 mg/kg/h	iv bolus or infusion	Asthma and overt heart failure are relative contraindications
Nicardipine	Calcium channel blocker	bolus: 30 mcg/kg up to 2 mg/dose infusion: 0.5–4 mcg/kg/min	iv bolus or infusion	May cause reflex tachycardia. Increases cyclosporine and tacolimus levels
Sodium nitroprusside	Direct vasodilator	Starting: 0-3-0.5–10 mcg/kg/min Maximum: 10 mcg/kg/min	iv infusion	Monitor cyanide levels with prolonged (>72 h) use or in renal failure; or co-administer with sodium thiosulfate
Useful for severely hypertensive patients with less significant symptoms				
Drug	**Class**	**Dose**	**Route**	**Comments**
Clonidine	Central α-agonist	2–5 mcg/kg/dose, up to 10 mcg/kg/dose given Q6–8 h	po	Side effects include dry mouth and drowsiness
Fenoldopam	Dopamine receptor agonist	0.2–0.5 mcg/kg/min up to 0.8 mcg/kg/min	iv infusion	Higher doses worsen tachycardia without further reducing BP
Hydralazine	Direct vasodilator	0.25 mg/kg/dose up to 25 mg/dose given Q6–8 h	PO	Half-life varies with genetically determined acetylation rates
Isradipine	Calcium channel blocker	0.05–0.1 mg/kg/dose up to 5 mg/dose given Q6–8 h	po	Exaggerated fall in BP can be seen in patients receiving azole antifungals
Minoxidil	Direct vasodilator	0.1–0.2 mg/kg/dose up to 10 mg/dose given Q8–12 h	po	Most potent oral vasodilator; long-acting

ACE angiotensin-converting enzyme, *BP* blood pressure, *IM* intramuscular, *IV* intravenous, *kg* kilogram, *mcg* microgram, *mg* milligram, *PO* oral with oral antihypertensives until the tumor can be surgically removed. Phenoxybenzamine, a potent α-adrenergic blocker, is usually recommended as the primary agent for this phase of management, although there has been a recent trend toward use of the peripheral α-adrenergic antagonist doxazosin for α-blockade [204, 205]. β-adrenergic blockade may be needed as adjunctive therapy to counter tachycardia once full α-blockade has been reached, especially in patients treated with phenoxybenzamine. Other important aspects of preoperative treatment include volume expansion and salt-loading; patients are typically admitted for 24–48 h prior to surgery to accomplish this. Patients who remain hypertensive postoperatively may require a short period of oral antihypertensive therapy; any agent is suitable in such cases. Patients with metastatic disease may require long-term antihypertensive therapy with either phenoxybenzamine or doxazosin.

Unanswered Questions/Research Needs

Despite the advances and the extensive literature on BP in children and adolescents, answers to relevant questions are still pending. The accurate measurement of BP is a prerequisite for the reliable diagnosis of HTN. Although automated electronic devices are currently recommended and widely used, the published evidence on their accuracy in children and adolescents is limited. Validated devices for oscillometric BP measurement should be developed, and inclusion of children in device validation studies should be encouraged. Concerted action of healthcare professionals, consensus organizations, industry, and society at large is needed for better identification of high BP identification and treatment among children and adolescents.

Robust reference values for office, ambulatory, and home BP, ideally including children and adolescents of multiple ethnic groups, need to be developed and matched with better knowledge about the development and progression of early organ damage. It is necessary to refine the clinical significance of white-coat HTN and masked HTN due to the discrepancies between office and out-off office BP. A better knowledge of all of these aspects may contribute to optimized interventions, preventing organ damage and improving long-term prognosis. Additional steps forward would be to obtain insights on central BP and pulse wave velocity as markers of function of the vascular system. Better understanding of isolated systolic HTN and its management is important in order to comprehend its significance and potential impact.

Preventive and treatment measures, such as healthy nutrition, low salt intake, nonsmoking, alcohol avoidance, and exercise activity, need to be encouraged. Clinical trials with antihypertensive drugs in order to gain knowledge about specific benefits and disadvantages of BP lowering agents and establish adequate doses should be designed and performed. Implementing electronic health technologies such as remote BP monitoring [206] will provide tools to improve attainment of BP targets and encourage adherence to prescribed therapies. Recognizing that the antecedents of adult HTN and its sequelae begin in childhood, the ultimate goal of all these efforts should be aimed at improvement in the long-term cardiovascular health of all individuals.

Cross-References

- ▶ Pathophysiology and Epidemiology of Hypertension in Children

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Renovascular Hypertension in Children: Evaluation and Management

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Kjell Tullus and Mieczysław Litwin

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Abstract

Renal artery stenosis is an unusual but important cause of high blood pressure (BP) in children. It often presents with very severely raised BP and symptoms of target organ damage. The cause is unknown in most cases, but fibromuscular dysplasia and Takayasu arteritis are the most common depending on the child's

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geographic location. A minority of cases are related to syndromes, mainly Neurofibromatosis type 1 and Williams syndrome. Ultrasound and CT angiography are used in the initial investigation, but digital subtraction angiography is the gold standard investigation. Medical treatment rarely achieves adequate BP control, and the most commonly used treatment is angioplasty. Vascular surgery such as arterial bypass or autotransplantation of the kidneys is used in a significant minority of children. Outcomes of angioplasty, including restoration of renal blood flow and improvement of BP, are good in the majority of children, but there is a significant minority where current treatment options are ineffective in achieving a sustained BP control and preventing end organ damage.

Keywords

Renovascular hypertension · Renal artery stenosis · Midaortic syndrome · Fibromuscular dysplasia · Takayasu arteritis · Neurofibromatosis type 1 · Williams syndrome · Digital subtraction angiography · Angioplasty · Vascular surgery

Introduction

In the majority of cases, hypertension (HT) is defined as primary, in the present-day situation of screening and the obesity epidemic. Of the secondary cases of HT, 5–10% are caused by renal artery stenosis (RAS). This chapter describes the etiology, investigations, treatment, and outcome of children with RAS.

Clinical Presentation

Renovascular hypertension (RVH) is diagnosed as an incidental finding in 27–54% of cases, with a higher frequency of incidental presentation in countries where blood pressure (BP) screening in children and adolescents is mandatory. RVH usually presents as severe hypertension, often fulfilling the criteria for the definition of hypertensive crisis. It is common in all age groups [1, 2]. Syndromic forms of RVH are accompanied by signs and symptoms typical for each clinical syndrome.

The clinical presentation of RVH is often nonspecific and may manifest as fatigue, reduced exercise capacity, or signs and symptoms directly associated with hypertensive crisis. Younger children usually do not complain of any symptoms or may present with nonspecific symptoms such as loss of appetite and failure to thrive [2, 3]. In some young children, the first presentation may be with signs of end organ damage and hypertensive crisis such as seizures, stroke, heart failure, lethargy, or failure to thrive. In contrast, older children more often present with a wide spectrum of neurological and cardiac signs and symptoms. Headache is the most commonly reported symptom and was recorded in 17–37% of patients [1, 2, 4–6]. In 18% of cases, headaches were accompanied by other symptoms, such as fatigue, vomiting and/or lethargy, and excessive screaming in younger children, which may be indicative of brain edema. Other neurological symptoms at presentation include seizures, eye ptosis, facial nerve palsy (7.7%), and stroke (subarachnoid, intracerebral, or ischemic; 3.9%). Ischemic stroke may be the first clinical symptom of neurovascular involvement of the external or internal cerebral arteries or of moyamoya disease which may be accompanied by renal artery stenosis. Other symptoms were epistaxis (5%), and in cases of unilateral renal artery stenosis hyponatremic hypertensive syndrome (HHS) with pressure natriuresis and polyuria (1–4%). Pulmonary edema was reported in children with bilateral renal artery stenosis [7]. Abdominal bruit was found in 10% of children. Cervical bruits were detected in 5.7% of children with RVH caused by fibromuscular dysplasia (FMD) [8] (Table 1).

Laboratory findings may indicate secondary hyperaldosteronism, especially in cases caused by unilateral renal artery stenosis (RAS), and may typically include hypokalemia with metabolic alkalosis. Urinalysis may reveal acidic urine. Plasma renin activity/renin concentrations and aldosterone levels are typically high; however, in cases of bilateral renal artery stenosis, renin generation and hyperaldosteronism may not be as strongly expressed due to sodium retention. Hyponatremia may be observed in cases of HHS with severe hypertension and pressure natriuresis. In unilateral renal artery stenosis, hypertensive injury to the contralateral kidney that is exposed to a high systemic BP may result in trace to severe proteinuria and red blood cells in the urine [9] (Table 1).

Features of hypertensive target organ damage are commonly found even at presentation. In reported series, left ventricular hypertrophy was noted in 56–67% of patients, and in 8% myocardial injury, manifesting as ischemia and/or rhythm disturbances, was observed [1]. Left ventricular hypertrophy was diagnosed more often in those who presented with clinical symptoms [1, 2, 10]. Subclinical arterial injury in the form of an increased carotid intima-media thickness was also commonly recognized, especially in cases with midaortic syndrome and renal artery stenosis [11]. Injury to the microcirculation manifesting as hypertensive arteriolopathy in the retina was found in 50–78% of patients [1, 10]. Narrowing of retinal arterioles (stage I) was detected in 47%, and arteriolar stiffening with venular compression (stage II) in 26.5% of patients. More severe forms of hypertensive retinopathy, namely stage III and IV, indicating damage to the microcirculatory barrier, were observed in 4.6% of cases [1].

Table 1 Presentation of renovascular hypertension in children

Presentation	%
Incidental finding or blood pressure screening	46–54%[a]
Headache	17–37%
Headache with vomiting, excessive screaming, and lethargy	11–23%
Heart murmur, congestive heart failure, and rhythm disturbances	8–10%
Hypertensive crisis (including seizures, Bell's palsy, ptosis, and stroke)	4–10%
Failure to thrive, weight loss	1–7%[b]
Epistaxis	4.5%
Symptoms caused by hypertensive injury to the kidney with patent renal artery:	
Proteinuria, hematuria	10%
Hyponatremic hypertensive syndrome	1–4%[b]

[a] More often in countries where blood pressure screening in childhood is mandatory

[b] More often in younger children, below 5 years

Etiology

The causes of renal artery stenosis (RAS) are several fold and presented in Table 2.

Fibromuscular Dysplasia

The underlying diagnoses causing RAS are not well defined in the majority of children, and in Europe and North America a majority of cases are given the diagnosis fibromuscular dysplasia (FMD) [2, 5, 7, 10, 12, 13]. This is a condition that can be diagnosed either from pathognomic "string of beads" patterns on renal angiography, or on histopathology [8]. Given that the majority of children with RAS are treated with angioplasty (see below), there is no opportunity to obtain a tissue sample for histological confirmation of the diagnosis.

Table 2 Causes of renovascular hypertension in children

Fibromuscular dysplasia
Syndromic
Neurofibromatosis type 1 (NF-1)
Williams syndrome
Alagille syndrome
Marfan syndrome
Other syndromes
Vasculitis
Takayasu's disease
Other systemic vasculitides
Extrinsic compression
Wilms tumor
Pheochromocytoma
Lymphoma
Other tumors
Other causes
Postradiation
Postumbilical catheters
Trauma
Rubella syndrome
Transplant renal artery stenosis

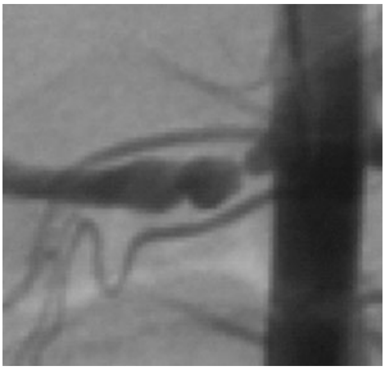

Fig. 1 Angiography in a child with renal artery stenosis due to fibromuscular dysplasia showing typical string of bead pattern

The string of beads pattern, Fig. 1, is a diagnostic feature in adult FMD but is only seen in a minority of cases in children, raising the question if adult and pediatric FMD are the same condition or not. FMD in children is thus mainly a diagnosis of exclusion without the possibility of confirming a positive diagnosis. Current classification of FMD used in adults distinguishes focal, multifocal, and multivessel lesions (Table 3); this has not been validated in children.

Syndromic RAS

A number of syndromes are associated with RAS. Of those, Neurofibromatosis type 1 and Williams syndrome are the two most common, accounting for 15–25% of cases in most published series [15]. Importantly, only a minority of children with these syndromes develop clinically overt RVH.

Takayasu Arteritis

Takayasu arteritis (TA) is the most common diagnosis given to children with RAS in Asia and Africa [16–19]. Takayasu arteritis is diagnosed with one obligatory criterion – an angiographic abnormality of the aorta or its main branches, plus at least one of the following four features: decreased peripheral artery pulses and/or claudication of extremities, BP difference between arm and leg of greater than 10 mmHg, bruits over the aorta and/or its major branches, hypertension, or a single value of ESR above 20 mm/h or CRP raised above normal range [20]. The authors emphasize that these are *classification criteria* thus indicating that a diagnosis of an inflammatory condition needs to be confirmed before using them. In many cases series of children with TA, more than half of the cases are diagnosed without any inflammation and the children are deemed to be in the late “burned out” phase [21]. It is unclear how the classification criteria can be used in these children.

Is It Fibromuscular Dysplasia or Takayasu Arteritis?

The differential diagnosis between FMD and TA remains unclear and controversial [15], and the reason for the difference in diagnostic patterns between countries is not fully known.

If we apply the criteria for FMD and TA, the vast majority of children in our renovascular clinics who do not have a clear syndrome or other specific etiology will fulfill the criteria for both conditions. This is obviously confusing and may lead to children not getting appropriate treatment. While it is very important that children with clear signs of an inflammatory vasculitis receive appropriate immunosuppression, it is also equally important that children without such symptoms and signs do not receive unnecessary treatment. Of note, appropriate anti-inflammatory treatment may not improve either the vascular lesions or the hypertension. Thus, in children with TA, when the vasculitis is under medical control, appropriate treatment of their vascular stenosis with either angioplasty or surgery is required.

Vascular Involvement

Renal artery stenosis can often be a condition involving many arterial beds. This seems to be the case regardless of the underlying etiology. Children can present with bilateral RAS in 53–78% of cases, midaortic syndrome in 48%, and stenoses of superior and inferior mesenteric

Table 3 Definition and classification of fibromuscuar dysplasia (FMD) according to First International Consensus on the diagnosis and treatment of fibromuscular dysplasia

Definition	FMD is an idiopathic, segmental, nonatherosclerotic, and noninflammatory disease of the musculature of arterial walls, leading to stenosis of small and medium-sized arteries. Histopathological classification is no longer clinically applicable. Current definition is based on radiographic criteria
Classification	FMD may result in two types of changes found on radiography: Focal FMD: May be found in any part of the artery Multifocal FMD: Areas of stenoses and dilation (string of beads) "Multivessel disease" is not included in the classification but is used in practice

Adapted from Ref. [14]

arteries in 53% [22]. Nevertheless, more frequent use of computed tomography angiography (CTA) in hypertensive children suspected of having RVH may lead to different results. However, more detailed, recent analysis of Assessment of Renal and Cervical Artery Dysplasia-Poland (ARCADIA-POL) Registry data has revealed that single-site FMD was found in 69.8%; 25% of participants, both adults and children with FMD, had cerebrovascular bed involvement, and 9.5% of them presented with visceral arterial involvement [23, 24]. Subanalyses of data from pediatric patients with FMD revealed that 29% of them presented with multivessel disease including visceral, carotid, and/or intracranial arteries (unpublished data). Due to the systemic nature of FMD, diagnostic imaging should also include other arterial beds besides the renal arteries. Although there are no specific pediatric recommendations for diagnostic imaging in children with FMD, recommendations for adults with FMD include the imaging of renal, visceral, cervical, and intracerebral arteries [14].

Aneurysmal Disease

A small group of children with FMD show aneurysms both of the renal arteries and also other vascular beds including, the aorta and iliac arteries, at presentation. In a small group of children, aneurysms are the predominant problem without any stenotic lesions. In one study from Michigan, a total of 41 children with 61 aneurysms in a large number of different vascular beds were presented [25]. A significant minority of these patients had comorbidities that may be causative of the aneurysm such as trauma, Kawasaki disease, or Ehler Danlos type IV syndrome, while most of the patients seemed to belong to the same spectrum of conditions as FMD.

Diagnostic Imaging

Renal ultrasonography with Doppler flow measurements is the first-line imaging used for diagnosis of renal artery stenosis. It is generally believed that renal ultrasonography with Doppler flow measurements should be offered to all hypertensive children, younger children before puberty, and older children with stage 2 hypertension because of greater likelihood of secondary hypertension in younger children and in those with more severe hypertension [26]. The other noninvasive imaging procedures include renal scintigraphy, CTA, and magnetic resonance angiography (MRA) which are used as second-line diagnostic procedures. Angiography can be both diagnostic and therapeutic, with angioplasty performed during the same session. The extent of imaging procedures depends on the etiology of RVH and may also include other arterial regions such as the aorta and visceral arteries, as well as cervical and intracerebral arteries. A suggested diagnostic algorithm is shown in Fig. 2.

Ultrasound Imaging (Fig. 3)

There are two Doppler imaging techniques, namely direct and indirect method, which should preferably be used in conjunction for the evaluation of the renal arteries. The direct method is based on the assessment of flow in the renal artery. It is performed from the aorta to the renal hilum.

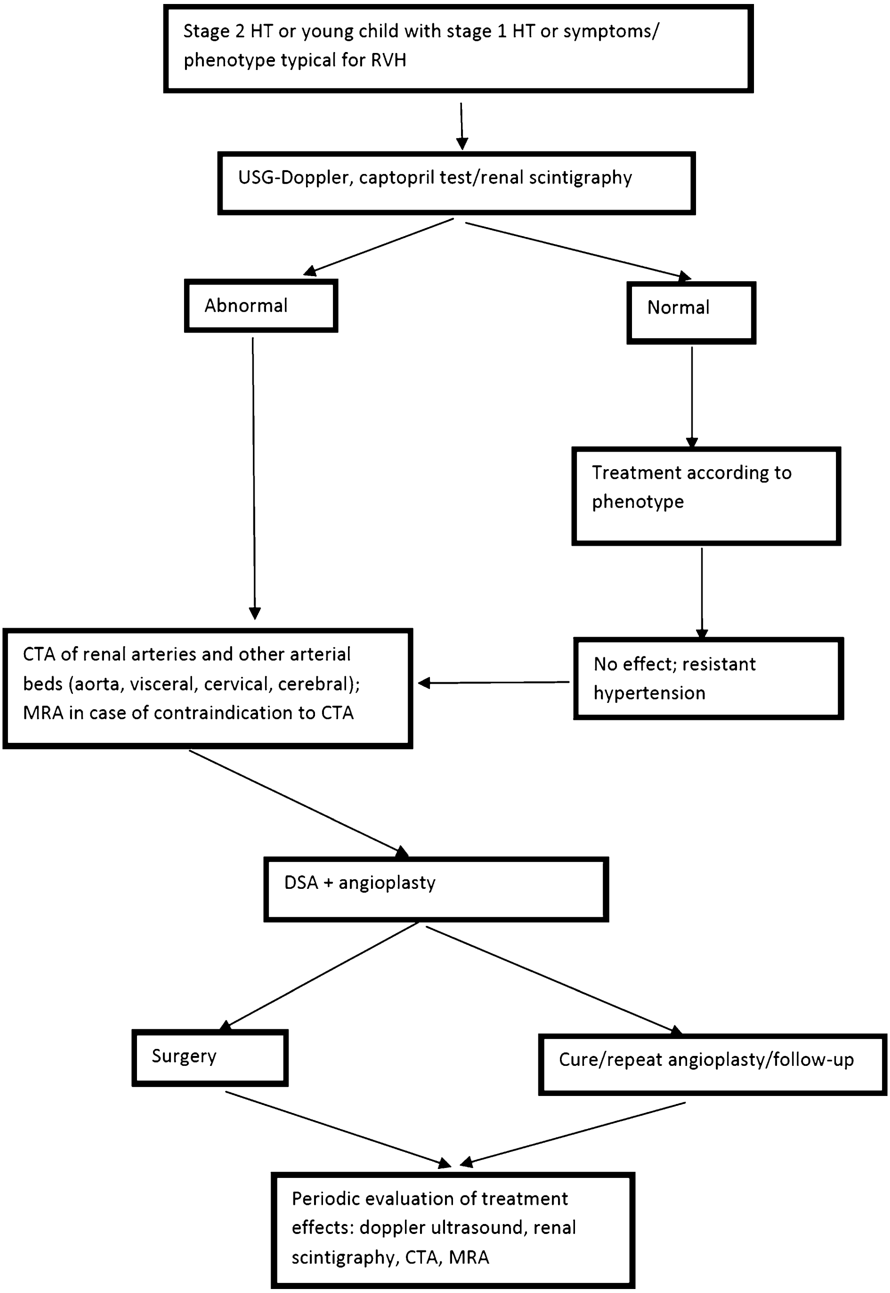
Stage 2 HT or young child with stage 1 HT or symptoms/ phenotype typical for RVH
USG-Doppler, captopril test/renal scintigraphy
Abnormal
Normal
Treatment according to phenotype
No effect; resistant hypertension
CTA of renal arteries and other arterial beds (aorta, visceral, cervical, cerebral); MRA in case of contraindication to CTA
DSA + angioplasty
Surgery
Cure/repeat angioplasty/follow-up
Periodic evaluation of treatment effects: doppler ultrasound, renal scintigraphy, CTA, MRA

Fig. 2 Scheme of diagnostic and management approach in children with renovascular hypertension. (Based on Refs. [22, 27])

The following parameters suggest hemodynamically significant renal artery stenosis:

- Peak systolic velocity (PSV) in the renal artery >180–200 cm/sec.
- Renal-aortic ratio (RAR = renal artery PSV/aortic PSV) >2–3.5.
- Renal-interlobar ratio (RIR = renal artery PSV/interlobar PSV) >5.

The indirect method is based on the assessment of the arterial flow in the renal parenchyma (either in the interlobar or arcuate arteries), with

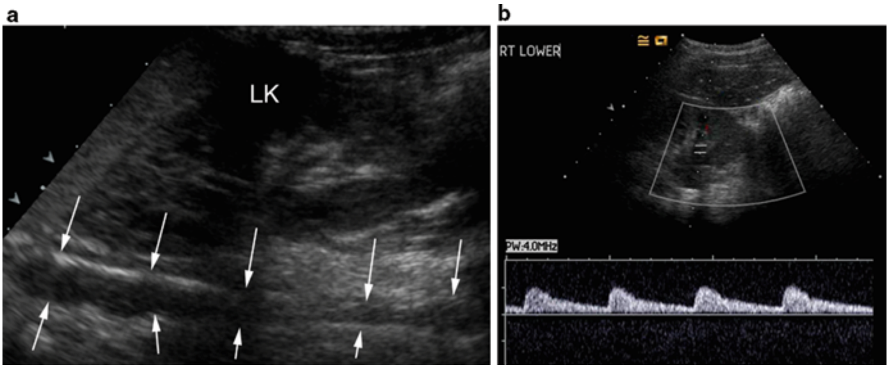

Fig. 3 Ultrasound showing. (**a**) midaortic syndrome (**b**) *parvus and tardus* flow pattern on Doppler imaging

the following abnormalities that may indicate renal artery stenosis:

- Presence of a *tardus et parvus* (slow and small) waveform (Fig. 3).
- Renal arterial resistive index (RI) <0.55.
- Difference between right and left kidney RI values $>5\%$.
- Acceleration time >70–80 milliseconds.
- Acceleration index (AI) <3 m/s^2.

Besides flow analysis, direct visualization of arterial stenosis or a significant (≥ 1 cm) difference in kidney length may suggest renovascular disease [28, 29].

Sonographic assessment of renal arteries is cheap, easily available, radiation free, and highly specific (94–99%). However, given that it is operator-dependent, and that the imaging quality may be affected by a lack of patient cooperation, it is characterized by low sensitivity (63–65%) for the detection of RAS. The sensitivity of ultrasonography in diagnosis of RVH can increase to 73% when kidney length is added to the analysis [29, 30]; however, its value is lower in case of peripheral, segmental branch stenosis [31].

Renal Scintigraphy

Renal scintigraphy, both static and dynamic, enables the assessment of tracer uptake and differential renal function. Scintigraphy captopril test is used to assess renal tracer uptake, after angiotensin-converting enzyme (ACE) inhibition, and may reveal abnormal blood flow to a kidney with a stenosed renal artery or its branches. Static renal scintigraphy with ^{99}technetium labeled dimercaptosuccinic acid (DMSA) shows only differential renal tracer uptake or its change after ACE inhibition. In contrast, dynamic renal scintigraphy with ^{99}technetium labeled mercaptoacetyl triglycine (MAG3) gives insight into blood flow to the kidneys, as well as into the velocity of intrarenal clearance and excretion. There is limited pediatric data showing that renal scintigraphy has a low sensitivity (48–86%) and specificity (73–92%). Therefore, renal scintigraphy is not recommended as a diagnostic test for RAS in children [26, 32, 33]. However, despite the above-mentioned limitations, this procedure is a useful tool in both the assessment of pre- and postintervention renal blood flow, as well as to define the differential renal function before intervention and at follow-up (Fig. 4a, b).

Magnetic Resonance Angiography (MRA) and Computed Tomography Angiography (CTA)

MRA is a noninvasive method of evaluating blood vessels, including renal arteries. Although it is regarded as attractive because of lack of radiation, this procedure requires patient cooperation such as breath holding and immobilization during scanning. In addition, MRA is time-consuming and requires sedation when performed in young children. Unenhanced (noncontrast) MRA has been found useful for visualizing renal

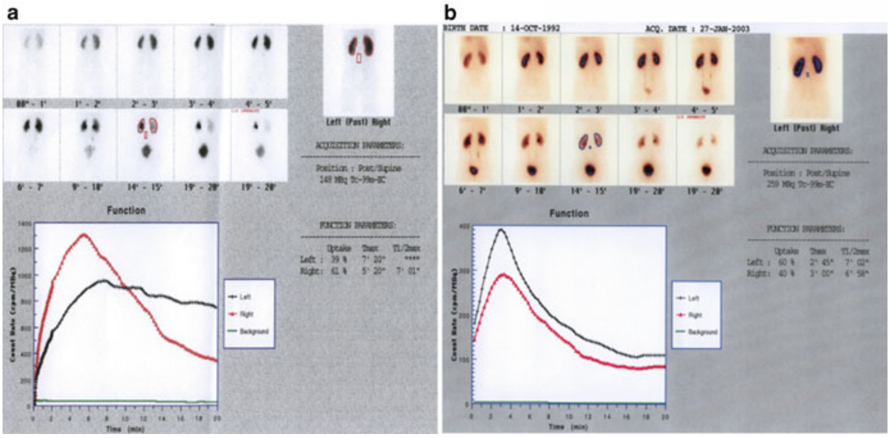
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Fig. 4 Dynamic renal scintigraphy: stenosis of main left renal artery. (**a**) Before angioplasty (**b**) After angioplasty

Table 4 Sensitivity and specificity of noninvasive imaging of renal arteries in diagnosis of renovascular hypertension. Comparison with digital subtraction angiography as reference method

	Sensitivity	Specificity
Doppler ultrasonography	73%	94–99%
Renal scintigraphy	48–86%	73–92%
Magnetic resonance arteriography	62.5–80%	63–100%
Computed tomography angiography	88%	81%

Adapted from Refs. [30, 31]

and visceral arteries in children. Additionally, the images are reported to be of the same or even better quality compared with contrast-enhanced MRA [34]. In adults, in whom the main cause of renal artery stenosis is atherosclerosis, contrast-enhanced MRA has a specificity and sensitivity of 97% and 93%, respectively [35]. In pediatric studies, nonenhanced MRA was found to have a sensitivity of 62.5–80% and specificity of 63–100% compared with DSA [30, 36].

In contrast to MRA, CTA acquisition times are very short, lasting only a fraction of a second, and the test provides good quality images. Thus, CTA can be performed even in small children without the need of general anesthesia. On the other hand, CTA requires administration of an iodine-based contrast agent and involves radiation exposure. It has higher sensitivity and specificity than MRA and better spatial resolution, which is especially important in the assessment of small intrarenal branches stenoses. Currently, MRA is recommended only when CTA is contraindicated [14]. In comparison with DSA as the reference method, CTA was found to have a sensitivity of 88% and specificity of 81% [30] (Table 4). With modern CT devices, the mean effective dose during CTA is between 3.9 and 6.3 mSv depending on the protocol, which corresponds to a radiation exposure related to 195–315 chest x-rays.

The real sensitivity and specificity of MRA and CTA is difficult to assess because the findings from these are usually the indication for referral for digital subtraction angiography (DSA). Given the low sensitivity and specificity of MRA and CTA in the diagnosis of RVH, it is important to maintain a low threshold for performing DSA even if MRA or CTA appears to be normal.

Digital Subtraction Angiography (DSA (Figs. 5 and 6)

As the sensitivity of CTA and MRA is relatively low (88% and 81%, respectively), DSA remains the gold standard in diagnosing RVH in pediatric patients with clinical and laboratory data suggestive of renovascular disease. Moreover, DSA allows for imaging of stenoses of small intrarenal,

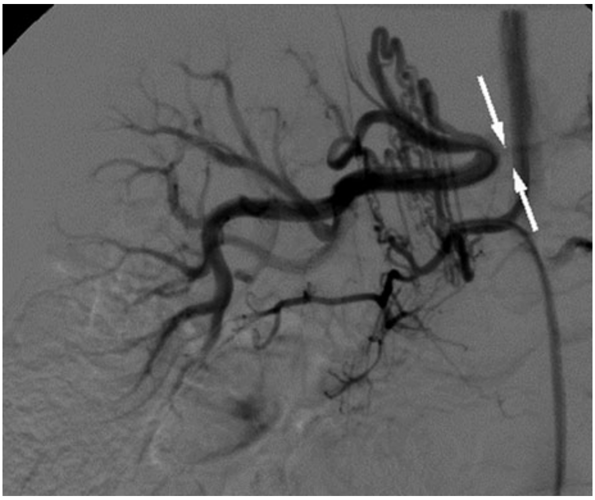

Fig. 5 Digital subtraction showing very significant right renal artery stenosis

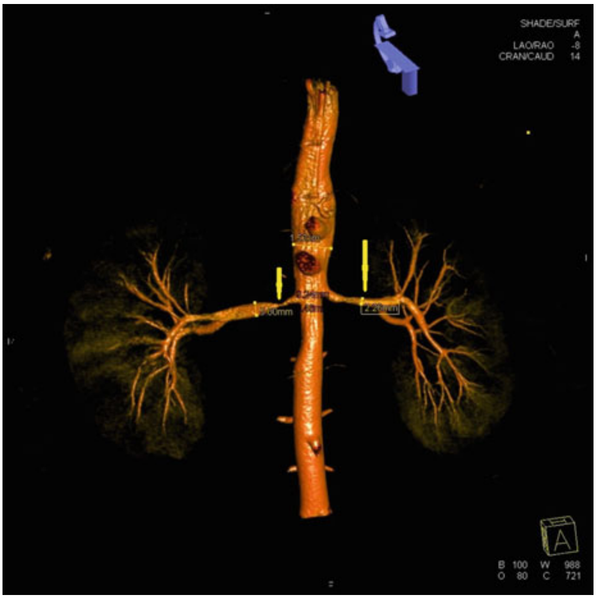

Fig. 6 Bilateral renal artery stenosis and midaortic syndrome on DSA

segmental branches of renal arteries which may be difficult to diagnose even with the use of modern, high-resolution noninvasive techniques. Because of its invasive nature, it is recommended that this procedure should be both diagnostic and therapeutic during the same session. DSA is recommended for finding the exact location and dimensions of the RAS that is suspected on noninvasive imaging such as Doppler ultrasonography and/or MRA/CTA. The other indication for DSA is severe hypertension without a clear cause identified by noninvasive imaging, especially when there is poor BP control with two or more antihypertensive medications [22].

The analysis of MRA/CTA findings makes it possible to prepare for angioplasty. The most commonly used method is the femoral approach; however, in some cases of anatomical variations of renal arteries or difficult femoral access because of stenosed or thrombosed femoral arteries, a subclavian, carotid, or even brachial approach may be used. In the situation where CTA is not performed before DSA, a cone beam CT can be performed at the start of the DSA study.

DSA requires administration of contrast usually, in a dose below 3 ml/kg body weight, but in difficult cases doses up to 6 ml/kg of body weight may be required [31].

Angiography permits making measurements of intravascular BP both proximal and distal to the stenosed segments. Intravascular ultrasonography is used in adults and makes it possible to visualize the arterial wall structure. However, there are only few pediatric reports on usefulness of this technique during angioplasty in children with stenoses of extrarenal arteries.

Renal Vein Renin Sampling

Renal vein sampling enables the assessment of renin generation by the whole kidney or its segments. This method is used when no evident stenosis is found on DSA and when distal stenoses of small branches of renal arteries are suspected. Blood samples are taken from the inferior vena cava both above and below the insertion of the renal veins, as well as from each renal vein and their intrarenal tributaries from upper, middle, and lower poles. Renin ratio 1.5: 1 between renal veins is considered as diagnostic and is supported by the finding of renin ratio below 1.3 between vena cava and contralateral renal vein. In case of intrarenal lesions, the renin concentrations in samples from different parts of the kidney are compared. Although it is believed that renin sampling may be of a greater significance in the diagnosis of ischemic part of the kidney in children than in adults, nowadays it is rarely performed.

Treatment

Treatment of RVH includes both medical and invasive therapy. Due to the complex nature of RVH and the need for a multidisciplinary approach, decisions about therapeutic management should be discussed by a team consisting of a hypertension specialist, interventional radiologist, cardiologist, and vascular surgeon. In children with RVH caused by arteritis, most commonly Takayasu arteritis, invasive treatment should be postponed until inflammatory activity is under control.

Medical Treatment

Most children with RVH present with severe hypertension and thus require antihypertensive treatment at diagnosis. However, medical therapy is only a bridge to invasive procedures such as angioplasty and/or surgery which are considered to be potentially curative. Bilateral RAS constitutes an absolute contraindication to ACEi/ARB treatment, but it is generally believed that neither ACEi nor ARB treatment should be used in children even with unilateral RVH since RAAS inhibitors may cause further ischemic injury to the already damaged kidney. It is, however, worth noting that renin-angiotensin aldosterone (RAAS) blockade with ACEi/ARBs is usually very effective at lowering BP. The mechanism of deterioration of function in the already ischemic kidney is not clear. It is assumed that RAAS blockade causes dilatation of the postglomerular efferent arterioles and, together with lowering of systemic BP, leads to an acute decrease in glomerular filtration.

There are no clear principles of pharmacological treatment of RVH. The treatment should consider presence of uni- or bilateral RAS, stenosis in other arterial beds, especially of intracerebral arteries, and electrolyte, and acid-base abnormalities caused by activation of the RAAS axis. Considering pathophysiological consequences of RAS, in the case of unilateral RAS, the contralateral kidney may react with pressure natriuresis, leading to subclinical dehydration. Thus, diuretics may aggravate sodium loss, and paradoxically, dehydration may lead to further stimulation of RAAS and BP rise. On the other hand, in the case of bilateral RAS, the activation of RAAS causes sodium retention thus making diuretics more effective.

Therefore, in our experience first-line treatment of unilateral RVH is based on a combination of a dihydropyridine calcium channel blocker, beta-adrenolytic without sympathetic activity, and alpha-adrenolytic. A mineralocorticoid receptor antagonist is used as the third or fourth line drug. Thiazide or loop diuretics are used as the last option. However, one must consider that sufficiently severe and long-lasting RVH caused by unilateral RAS may cause hypertensive nephropathy in the contralateral kidney with a patent renal artery that is persistently exposed to a high BP. Such kidney injury may lead to secondary sodium retention. In these cases, diuretics, prescribed as the second or third line drugs, are very effective. ACEi/ARBs are used in exceptional cases of unilateral RAS where other treatment options have failed. In case of bilateral RAS, diuretics, usually thiazides, combined with calcium channel blockers, beta- and alpha adrenolytics, are the first-line drugs. Mineralocorticoid receptor antagonists are also used.

In published series of children with RVH, the median number of antihypertensive drugs that were used were 3–3.4, but ranged from 1 to 8 [2, 37]. However, the efficacy of antihypertensive medical treatment of RVH was limited. Both our experience as well as the results of other groups showed that normotension was achieved only in 5–5.7% of cases, BP improvement in 21.6–43% of patients, while in 51.7–72.9% of patients, only a marginal improvement in BP was observed [1, 6]. Nevertheless, it must be noted that in most of these cases, neither ACEi nor ARBs were used.

Angioplasty

Percutaneous angioplasty is now regarded as the main form of treatment of RVH. There are several types of this procedure including balloon angioplasty, angioplasty with drug-coated balloons, cutting balloon angioplasty, and angioplasty with stents using self-expanding, bioabsorbable, and drug-eluting stents. The decision to perform angioplasty is based on the anatomical characteristics of the stenosed site obtained from CTA scans performed before or during angiography and confirmed by DSA findings. In general, ostial stenoses (i.e., stenosis at the origin of the renal artery from the aorta) are difficult to dilate and, according to some authors, are an indication for surgical treatment [38]. However, in many centers angioplasty is tried first and surgery is considered only in case of unsuccessful interventional radiology procedure.

Balloon angioplasty usually serves as the first choice procedure, but it may also be performed when reintervention is required or as a rescue therapy when complications of a procedure occur. The balloon diameter is chosen based on the diameter of renal artery proximal to the stenosed site. In recent years, angioplasty with drug-coated balloons is performed more and more frequently, usually during reintervention and with encouraging results [37]. Cutting-balloons are used in case of long segment stenosis or when there is a fibrosed area of the vessel wall such that the artery cannot be dilated with the balloon alone. Stents are generally avoided in children because of the risk of stenosis that can develop as the child grows. There is also a greater risk of in-stent stenosis caused by intimal hyperplasia and/or thrombosis. However, with increasing experience with stenting of other arterial beds and use of drug-eluting, self-expanding, and bioabsorbable stents, they are used more commonly even in children with RVH. According to recommendations, stents should be avoided in RVH in patients with FMD. However, they are used as rescue treatment in case of complications during balloon angioplasty such as dissection or severe contraction of the renal artery [14].

Angioplasty leads to normalization or improvement of BP control in 50% and, in some publications, even up to 100% of cases. However, some patients need more than one angioplasty procedure due to inadequate vessel dilatation or restenosis. In our experience, two procedures were required in 18–25% of cases and three angioplasties in 10–12% of patients.

Embolization

Embolization is considered in case of RVH caused by stenosis of segmental or subsegmental renal arteries that are not amenable to angioplasty or surgical revascularization. The embolizing substance is ethanol which causes endothelial injury and infarction of the part of the renal tissue supplied by the stenosed vessel. The loss of renal tissue is lower than with partial nephrectomy. Usually, there is no need for surgical nephrectomy/partial nephrectomy after procedure, but pediatric experience is still limited [39, 40].

Surgery

There are two types of surgical treatments: nephrectomy and revascularization. Nephrectomy is indicated in case of a poorly functioning ischemic kidney, usually with split (single kidney) GFR below 10%. However, case reports indicate that even poorly functioning kidneys may significantly improve their function after revascularization; microscopic findings have revealed that normal glomerular and tubular structure may be present in the ischemic kidney [41].

Surgical revascularization procedures are usually considered the second step after an unsuccessful endovascular intervention. An exception to this is tight ostial stenosis, wherein surgical revascularization may be considered first-line treatment. The main revascularization procedures used in children with RVH are kidney auto-transplantation (Fig. 7), renal artery reimplantation, and different kinds of bypass procedures with the use of autologous veins or synthetic grafts (Fig. 8).

Sympathetic Renal Denervation (SRD)

Sympathetic renal denervation (SRD), both surgical and endovascular, is used in treatment of resistant hypertension in adults. Although there is strong evidence from animal studies that afferent renal denervation leads to significant lowering of BP in experimental models of renovascular hypertension, SRD was not used in patients with RVH. There are, however, case reports of successful SRD in one adolescent with severe hypertension after surgical repair of aortic coarctation [42] and in a girl with Turner syndrome [43].

Outcome

The outcome of children with RVH may be recorded in several ways. The most common measure is anatomical success and improvement in BP. Many studies present results in the short or medium term. However, a significant proportion of children with RVH have a progressive condition and may, even after an initially successful procedure, represent with hypertension and need further treatment. The pathophysiology of this process is not well defined.

The greatest success of angioplasty is noted in cases with focal, uni-, or bilateral stenosis of the main renal artery with 40–85% of patients achieving normotension after angioplasty [2, 5]. In the case of RAS and intrarenal disease and/or mid-aortic syndrome, the success rate is lower and ranges from 0 to 25%; however, some improvement in BP was noted in 41.5% (25–58%) of cases [1, 2, 5, 37]. In general, single centers reports

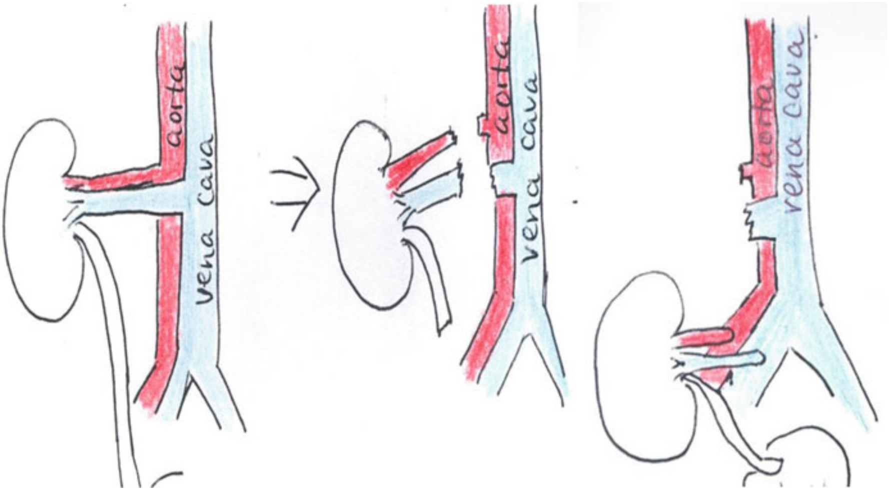

Fig. 7 The principle of autotransplantation

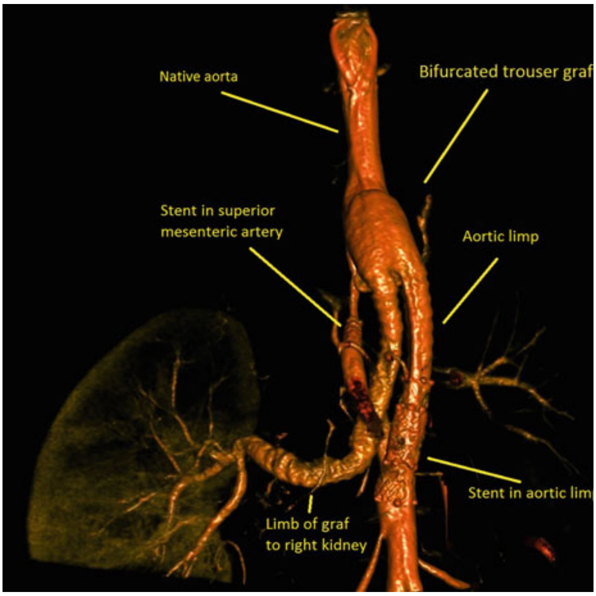

Fig. 8 Severe midaortic syndrome and bilateral renal artery stenosis treated surgically with a "trouser graft"

indicate that the median number of antihypertensive drugs prescribed decreased from 3 (1–6) to 1.3 (0–5) as a result of angioplasty. However, the procedure was ineffective in 7–23% of patients [1, 2, 31, 37].

The reported success rate of endovascular procedures appears to be similar in patients with NF1 and those presenting other etiologies. Thus, their effectiveness is thought to be related to the localization and extent of the stenotic lesion rather than to the etiology of RVH. Similarly, in patients with Takayasu arteritis, the success of angioplasty and stenting seems to be lower than in cases of other etiologies. This might be related to the extent of aortic changes rather than to the underlying disease.

The group in Michigan has the largest experience with surgery in these children. Their most recent report, using mainly classical vascular surgery techniques such as renal reimplantation to the aorta, aortorenal bypass, patch aortoplasty, and aortic bypass, shows results that are not dissimilar to those of most series on angioplasty [44]. Autotransplantation of the kidneys is a surgical method that seems to gain more and more popularity in many centers, and the short- and medium-term effects of auto-transplantation are good with up to 30–43% of patients "cured" (i.e., without a need for antihypertensive medications), and 41–50% of achieving normotension with antihypertensive drugs [45].

The outcome regarding long-term kidney function is less well reported. This should include both overall kidney function and the function of each kidney. Most series, including ours, have not reported any child who has lost both kidneys, but in a small group of patients one kidney may have to be sacrificed either through nephrectomy or because it was impossible to improve that kidney's blood flow.

A proportion of, mainly small, children have very severe heart involvement at presentation. In most cases, this seems to improve with improving BP, but very few reports have systematically looked into this issue. A surprisingly small group of children with RAS and concomitant midaortic syndrome have significant claudications. The long-term outcome of this is not fully known.

As with many childhood conditions, the transition to adult care is an important process that requires careful planning. We suggest that pediatric centers specializing in the care of children with RVH develop a long-term relation with an adult center that can take over the care of children who are likely to need long-term follow-up.

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Part X

Acute Kidney Injury

Pathogenesis of Acute Kidney Injury

65

David P. Basile, Rajasree Sreedharan, Rajit K. Basu, and Scott K. Van Why

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Abstract

The rapid loss of kidney function that results from renal ischemia-, sepsis-, toxic-, or toxicant-induced renal cell injury is termed acute kidney injury (AKI). The operative word “injury” initially referred primarily to renal tubule cell injury. However, it has since become clear that the expanse of cellular alterations and injury is broad, affecting other types of cells resident in the kidney that are central to the pathophysiology of AKI. This chapter focuses on the pathophysiology of AKI triggered by an ischemic insult as revealed in experimental models both in vivo and in vitro. How mechanisms of sepsis-induced AKI, a principal cause of AKI in children, overlap with and differ from ischemia-induced AKI is discussed. Included are classical concepts of acute tubular necrosis as well as a contemporary understanding of vascular, cellular, molecular, inflammatory, and metabolic alterations that are associated with renal cell injury. Mechanisms that lead to cell injury and death are addressed along with processes that can result in cellular repair and renal recovery.

Keywords

Acute kidney injury · Ischemia · Sepsis · Inflammation · Cell death · Hemodynamics · Microvessels

Introduction

The sudden loss of renal function that may result from inadequate renal perfusion, arterial or venous obstruction, renal cell injury, or acute obstruction to urine flow historically was termed acute renal failure (ARF). Well over a decade ago the term acute kidney injury (AKI) was proposed to replace the term ARF [1]. Usage of “AKI” is now nearly uniform. Unfortunately, it is often used imprecisely, defeating the purpose of the change in nomenclature.

AKI was not meant to be synonymous with the old term ARF, nor the associated more narrow term “acute tubule necrosis” (ATN). The intent of the change was to standardize the definition to focus on the operative word “injury,” highlighting the spectrum of injury and mechanisms that underlie the attendant renal dysfunction. Embedded in the change in terminology was the goal to improve translation of knowledge from basic science studies to clinical studies of AKI by defining affected patients more precisely. In so doing, evaluation of potential biomarkers as predictive of severity of renal injury and outcomes could be enhanced [2]. In addition, well-defined target populations could then be chosen for translational studies of potential treatment modalities, identified in basic science studies of AKI, that had been found to limit injury or enhance recovery of injured renal cells from a specific insult.

Initially "injury," the unifying feature of AKI, was understood to be primarily renal tubule cell injury. However, it has since become clear that the expanse of cellular alterations and injury is broad, affecting other types of cells resident in the kidney that are central to the pathophysiology of AKI. The clinical manifestations of AKI range from mild but sustained elevation in serum creatinine (renal insufficiency) to anuric renal failure. In contrast, rapidly and fully reversible causes of acute renal *insufficiency*, such as from acute and reversible cardiac insufficiency or volume depletion, are specifically excluded from the spectrum of AKI [1]. Thus, the use of the term "pre-renal AKI," now rather common, is both nonsensical and antiquated, effectively reverting back to the old imprecise term ARF and accompanying terms such as "pre-renal ARF." More precise and clear, then, is to keep the term AKI separate from potentially rapidly reversible pre-renal causes of acute renal insufficiency. As intended, this can facilitate improved patient care and targeted clinical studies of AKI separate from rapidly reversible causes of renal insufficiency. The review of the pathogenesis of AKI in this chapter, then, is restricted to this precise definition.

Renal cell injury results from an ischemic, toxic (e.g., myoglobin), or toxicant (e.g., aminoglycoside) insult that causes acute tubular damage with accompanying loss of ability to reabsorb filtered solute. The resulting decrease in glomerular filtration rate (GFR), an invariable component of AKI, is then a successful adaptive response, since continued filtration of plasma across the glomerular basement membrane without reabsorption of the filtrate by injured renal tubules would result in massive losses of salt and water [3]. Thus, the decreased GFR associated with AKI prevents severe depletion of extracellular fluid.

Renal ischemia is both the most common isolated cause of AKI, and a contributor to multifactorial AKI, such as in sepsis syndrome with multiorgan dysfunction. For decades, then, studies of AKI have focused on models of ischemic renal cell injury. From these studies it is clear that AKI manifestations can range from mild, sublethal cell injury with disrupted renal cell architecture and function to fulminant necrotic cell death (i.e., ATN). Furthermore, both extremes, along with the full spectrum of intervening cell injury features, can be present simultaneously or sequentially after the insult (Fig. 1).

This chapter, then, focuses on the pathophysiology of AKI triggered by an ischemic insult as revealed in experimental models both in vivo and in vitro. How mechanisms of sepsis-induced AKI, the most common cause in children, overlap with and differ from ischemia-induced AKI is discussed. Included are classical concepts of ATN as well as a contemporary understanding of vascular, cellular, molecular, and metabolic alterations that are associated with renal cell injury. Mechanisms that lead to cell injury and death will be addressed along with processes that can result in cellular repair and renal recovery.

Phases of Acute Kidney Injury

Clinically, AKI can be divided into initiation, extension, maintenance, and recovery phases. These clinical phases directly relate to cellular events that occur during the injury and recovery process (Fig. 2). The initiation phase of AKI occurs when renal blood flow decreases to a level causing severe cellular ATP depletion that in turn leads to acute renal tubule cell injury and dysfunction [4]. Renal ischemia rapidly induces a number of structural and functional alterations in renal proximal tubule cells that are directly related spatially and temporally with disruption of the normal framework of filamentous actin (F-actin) [5–7] (described later in this chapter). The extent of these alterations depends on the severity and duration of ischemia. Although these alterations may fall short of being lethal to the cell, they disrupt the ability of renal tubules to maintain normal function. In addition, ischemic injury to vascular smooth muscle and endothelial cells during the initiation phase contribute to the structural abnormalities observed in the renal vasculature in ischemic AKI [8–10]. Activation of epithelial and possibly endothelial cells during the early initiation phase induce a variety of chemokines and cytokines that are instrumental in initiating the

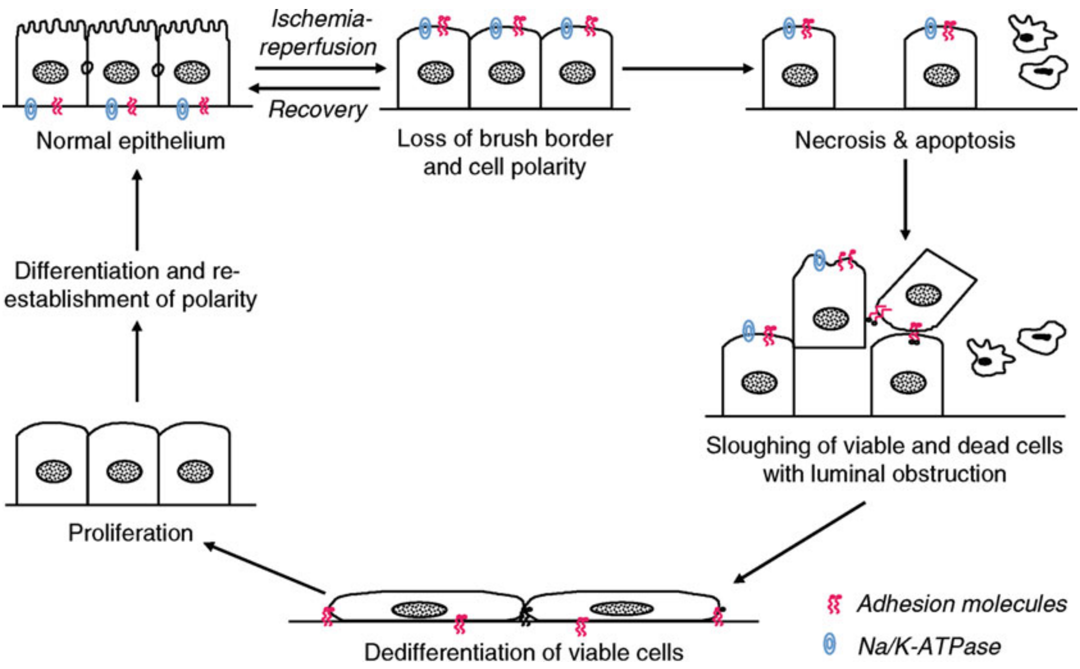
Ischemia-reperfusion
Recovery
Normal epithelium
Loss of brush border and cell polarity
Necrosis & apoptosis
Differentiation and re-establishment of polarity
Sloughing of viable and dead cells with luminal obstruction
Proliferation
Dedifferentiation of viable cells
Adhesion molecules
Na/K-ATPase

Fig. 1 *Disruption and recovery of renal tubule architecture*. Ischemia disrupts normal polar distribution of membrane transport proteins and adhesion molecules, causing loss, respectively, of reabsorptive function and tubule integrity. Sublethally injured cells can recover normal structure and function. More severely injured cells die of apoptosis or necrosis, resulting in denudation and obstruction of the tubule. Restoration of tubule structure proceeds through dedifferentiation, proliferation, and redifferentiation processes likely mediated by growth factors and stem cells

inflammatory cascade ([11, 12] and see below). The cellular localization of these phenomena within the kidney continues to be elucidated.

The extension phase, which occurs during reperfusion, is ushered in by continued regional hypoxia along with triggering of an inflammatory response (Fig. 2). Both events are more pronounced in the corticomedullary junction (CMJ), or outer medullary region, of the kidney [11, 13]. It is during this phase that renal vascular endothelial cell damage appears to play a central role in the continued ischemia of renal tubules, as well as in the accompanying inflammatory response. Tubule cells continue to undergo injury and death, with both necrosis and apoptosis occurring predominantly in the outer medulla [14]. In contrast, the proximal tubule cells in the outer cortex, where blood flow has returned to near normal levels, undergo cellular repair during this phase. As cellular injury continues in the CMJ region during the extension phase, the GFR continues to fall. There is continued production and release of chemokines and cytokines that further enhance the inflammatory cascade [15]. The extension phase is thought to represent the most promising phase for successful therapeutic intervention in ischemic AKI. Leukocytes infiltrate as early as 2 h, and inflammatory cell infiltration in the outer medullary region of the kidney is significant by 24 h following ischemia [16]. So, treatments designed to interrupt the inflammatory cascade might attenuate the cellular injury and attendant loss of renal function during this phase.

During the clinical phase known as maintenance, cells undergo repair, migration, apoptosis, and proliferation so as to maintain and restore cellular and tubule integrity (Fig. 2). GFR is stable, albeit at a lower level determined by the severity of the initial event. This cellular repair and reorganization phase results in slowly improving cellular function and sets the stage for improvement in global organ function. Blood flow returns toward normal, and epithelial cells begin to restore intracellular and intercellular

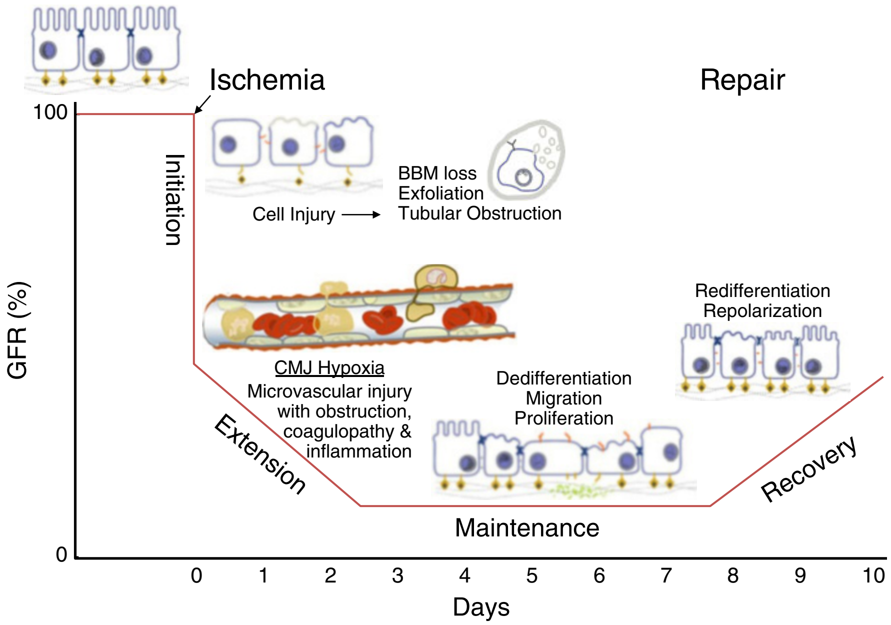
Ischemia
Repair
100
0
GFR (%)
Initiation
Cell Injury
BBM loss
Exfoliation
Tubular Obstruction
CMJ Hypoxia
Microvascular injury
with obstruction,
coagulopathy &
inflammation
Extension
Dedifferentiation
Migration
Proliferation
Redifferentiation
Repolarization
Recovery
Maintenance
0
1
2
3
4
5
6
7
8
9
10
Days

Fig. 2 *Relationship of cellular to clinical phases of AKI.* Impaired renal blood flow causes the initiation phase with onset of cellular injury, particularly in the proximal tubule (*BBM* – brush border membrane). The extension phase is manifested by microvascular injury triggering inflammatory processes and causing ongoing corticomedullary junction (CMJ) hypoxia. During the maintenance phase, GFR reaches a nadir as cellular repair begins. The recovery phase is marked by progressive cell and organ structural and functional recovery, with concomitant improvement in GFR. (From Ref. [19])

homeostasis. During the recovery phase, return of cellular differentiation, epithelial polarity, and normal cellular and organ function is achieved.

Renal Hemodynamics

Renal vasoconstriction is intense in experimental and clinical AKI [17]. Because vasoconstriction was considered to be the dominant factor, it was suggested long ago that the term "vasomotor nephropathy" might be more appropriate than acute renal failure to describe this condition [18]. The hypothesis was that an insult to the renal tubular epithelium results in release of vasoactive compounds that increase cortical vascular resistance causing a decreased in renal blood flow (RBF), thus perpetuating injury to the tubule and furthering the cycle. Release of vasoconstrictive compounds may then diminish GFR by constricting afferent and efferent arterioles, thereby causing diminished urine output, or oliguria. Candidates for the vasoconstrictive components of AKI include angiotensin, metabolites of arachidonic acid, adenosine, endothelin, and nitric oxide.

Renin-Angiotensin System

The renin-angiotensin system (RAS) functions in a manner consistent with the renal vasoconstriction hypothesis. In the setting of volume depletion, reduction in renal perfusion may cause pre-renal azotemia. Prolongation of this state may result in frank parenchymal tissue damage, i.e., AKI [19]. Under these conditions, compensatory activation of the RAS system would be expected to maintain volume and perfusion pressure. In addition, because Ang II is thought to constrict preferentially efferent arterioles, its activity may be adaptive for preserving GFR in pre-renal conditions.

There is ample evidence of an increase in both local and circulating RAS components (angiotensinogen, renin, Ang II as well as other Ang-derived peptides) in patients experiencing AKI or animal models of AKI [20]. Whether the RAS participates in renal vasoconstriction leading to AKI and whether this system should be targeted clinically are questions that have failed to garner any consensus. Several studies have investigated whether the RAS contributes to the development of AKI. For example, in an animal model of ischemia/reperfusion, pretreatment with the Ang II type 1 receptor antagonist losartan prevented renal injury [21]. However, initiating hemodynamic parameters were not evaluated in the study, with the suggestion that the results may have been due to Ang II-mediated effects on inflammation. Despite such studies over many years, the role of the renin-angiotensin system as the modulator of renal injury in AKI remains uncertain.

Nevertheless, interest in this pathway remains since it appears that aldosterone, downstream from renin and angiotensin, may play a central role in ischemia-induced AKI [22]. Prior mineralocorticoid receptor (MR) blockade with spironolactone was found to prevent the typical reductions in RBF and GFR during reperfusion after ischemia, and substantially reduced accompanying renal histomorphologic damage and tubule apoptosis. The protection conferred by MR blockade correlated with changes in other pathways that have been implicated in AKI. Primarily, it appeared that MR blockade decreased oxidative stress present during reperfusion after renal ischemia.

Arachidonic Acid

Arachidonic acid (AA) is released from membrane phospholipids in the renal outer medulla in response to ischemia. AA can then be metabolized by several enzymes to produce various vasoactive compounds. One pathway is through cyclooxygenases to produce prostaglandins. Several prostaglandins are potent renal vasodilators. While inhibition of prostaglandin production is associated with decreased RBF and can affect the severity of AKI, the precise role of prostaglandins in the induction and maintenance of AKI is not established [6]. However, prostaglandin inhibitors, such as nonsteroidal anti-inflammatory drugs, increase the risk for development of AKI and may act synergistically with other insults. AA can also be metabolized to 20-hydroxyeicosatetraenoic acid (20-HETE) by cytochrome P450 ω-hydroxylase. In the kidney, 20-HETE modulates vascular tone, RBF, and sodium transport in the tubule. Further, 20-HETE has a demonstrated role in ischemic AKI [23]. Ischemia induces a dramatic increase in 20-HETE in the rat kidney and inhibition of 20-HETE synthesis worsens AKI. Administration of 20-HETE analog ameliorates ischemic AKI and prevents the typical postischemic decrease in renal medullary blood flow in rat kidneys. So, 20-HETE may mitigate AKI by preventing postischemic medullary hypoxia by increasing renal medullary blood flow [23].

Adenosine

Adenosine, which results from catabolism of adenine nucleotides, is a potent vasoconstrictor of renal vasculature, whereas it is a vasodilator of peripheral vasculature [24]. The concept that adenosine is an important vasoactive compound following an acute tubule insult is attractive, since it would link alterations in cell metabolism with hemodynamic changes. However, the evidence that adenosine is a major factor in the vasoconstrictive response in AKI is inconclusive. There is evidence that methylxanthines (an inhibitor of adenosine receptors) or the adenosine A1 receptor antagonist DPCPX improves renal function in the early phases of ischemia/reperfusion injury [25]. However, whether endogenous adenosine contributes to renal hemodynamic impairment in AKI is not clear. Adenosine levels are reduced during reperfusion injury by adenosine deaminase, despite persistent renal vasoconstriction [26]. Mice with a knockout mutation of the adenosine A1 receptor (thought to mediate tubuloglomerular feedback) had unexpectedly worse ischemic renal injury than wild-type mice, and exogenous activation of A1AR was protective [27]. The data, then, suggested that tubuloglomerular feedback through A1AR may be an adaptive response in AKI that limits delivery of solute to proximal tubules and thereby reduces the metabolic demand on susceptible, injured tubule cells.

Endothelin

Endothelin, among its various biological activities, is a potent renal vasoconstrictor [28–31]. Endothelin conveys its effects via at least two distinct receptor subtypes, the ET-A and ET-B receptors. Activation of either receptor has the potential to reduce GFR. Raised endothelin levels in plasma and in both the cortex and medulla of the kidney are found in experimental animals after induction of renal ischemia [28]. In a rat model of cold ischemic storage and transplant, preproendothelin mRNA was upregulated and immunoreactive ET-1 localized in the peritubular capillary endothelial cells following reperfusion injury [32].

The postischemic infusion of antiendothelin antibody or endothelin receptor antagonists provides protection from ischemic injury in animal models [28–30, 33, 34]. Selective ET-A antagonism inhibits afferent arteriolar constriction, while specific activation of ET-B resulted in both afferent constriction as well as efferent vasodilation [35]. In humans, there is evidence that the endothelin system may be activated in the setting of acute kidney injury. Increased endothelin levels are found in patients with AKI due to severe sepsis [36]. However, the effect of ET antagonism in humans is less clear, as it did not afford protection against contrast-induced nephropathy [37].

Nitric Oxide

Nitric oxide (NO) is a potent renal vasodilator, which is widely distributed in the kidney, and is produced from L-arginine by constitutive and inducible nitric oxide synthetases (NOS) located in endothelial cells and renal tubules [38]. The effect of either stimulation or inhibition of NOS on ischemic or toxic AKI is confusing and, at times, contradictory. The apparent contradictory effects are likely attributable to the differential distribution and specific effects of NOS isoforms on either the renal vasculature or renal tubules.

NOS3 (endothelial NOS) is produced at high levels particularly in the renal medulla [39] and its activity may represent an important mechanism to preserve medullary blood flow in response to renal vasoconstrictors such as Ang II [40]. Following renal injury, there is impaired endothelial NOS function with loss of vasodilator responses to acetylcholine and bradykinin [41]. A role for NOS3 in preserving perfusion in AKI is suggested by the greater degree of renal damage that occurs in NOS3 knockout mice in response to endotoxin [42]. Animal models have also used approaches to increase or preserve renal NO activity. For example, infusion of the precursor L-arginine, inhibition of enzyme arginase-2, infusion of NO donors, or the NOS3 cofactor tetro-hydrobiopterin can preserve renal vascular perfusion and attenuate AKI induced by ischemia [43, 44]. Clinical studies have suggested that the use of inhaled NO gas can reduce the risk of AKI in patients undergoing cardiopulmonary bypass surgery [45]. In addition, lowering of asymmetric dimethyl arginine (ADMA), thought to be an endogenous eNOS, by infusion of a novel recombinant ADMA metabolizing enzyme was recently shown to protect against ischemic AKI in rats [46].

In contrast, NOS2 (inducible NOS), either by promoting inflammation or oxidative stress, may be deleterious in AKI. Nonselective NOS inhibition was found to worsen renal function and cause profound renal vasoconstriction [47], while antisense oligonucleotides targeting NOS2 in rat kidney protected against ischemia-induced AKI [48]. Hence the dilemma: Preservation of NO in the renal vasculature via NOS3 could attenuate vasoconstriction and potentially lessen AKI; induction of NO via NOS in renal tubules is cytotoxic [47, 48]; and potential approaches to mitigate NOS should be careful to consider isoforms of NOS being targeted.

All things considered, persistent renal vasoconstriction is a well-documented event during reperfusion following renal ischemia, but once AKI has been established, the infusion of potent vasodilators such as prostaglandins or dopamine does not lead to sustained improvement in GFR [49, 50]. Confirming the findings of animal studies, several human trials of renal vasodilators such as dopamine have failed to demonstrate improvement in GFR in established AKI despite augmentation of total renal blood flow [51]. Although renal hemodynamic factors play an important role in initiating AKI, alterations in renal vascular resistance and renal perfusion on a macroscopic scale may not be dominant determinants of renal epithelial cell injury. They may, in fact, be an adaptive response to ameliorate further cellular injury during reperfusion. On the other hand, microvascular alterations are now recognized to play a major role, as discussed below.

Alterations in Microvasculature

An old and consistent observation is medullary vascular congestion following experimentally induced ischemic renal injury. Advances in recent years, including application of in vivo microscopy, has given substantial credence to the concept that injury to the vascular endothelium is central to the initiation and extension of AKI. The microvascular endothelium undergoes direct structural alterations after an ischemic insult, and is both a source of and a target for inflammatory injury [52–56]. Endothelial damage in the setting of AKI includes alteration in actin cytoskeleton structure, loss of glycocalyx and barrier function, and evidence of swelling, blebbing, and exocytosis of Weibel-Palade bodies [19, 43, 57]. In combination with chemokines liberated from nearby damaged epithelial cells, the cumulative result of these events is postulated to promote infiltration and adhesion of leukocytes within the renal microvasculature.

Minimally invasive intravital microscopy in animals has revealed sporadic cessation and even reversal of blood flow in peritubular capillaries in the renal cortex during reperfusion [58, 59]. Near-infrared laser speckle blood flow imaging shows that AKI in animal models is associated with an increase in the heterogeneity of blood flow, which is independent of total organ perfusion [60]. Such heterogeneity may underlie the focal hypoxia that contributes to the extension of renal injury during reperfusion. Administration of fully differentiated endothelial cells into postischemic rat kidneys ameliorated these early alterations in microvascular flow and the subsequent loss of GFR [59]. Similar but less impressive amelioration of AKI was achieved using surrogate cells expressing endothelial nitric oxide synthase (eNOS).

It has been suggested that alterations in microvascular perfusion patterns occur, in part, as a result of microvascular leukocyte adhesion. Ischemic AKI increases endothelial expression of a variety of adhesion molecules that promote endothelial-leukocyte interactions. These include intercellular adhesion molecule-1 (ICAM-1), P-selectin, and E-selectin [53, 61]. Ablation of the ICAM-1 gene or pretreatment with ICAM-1 antibody rendered mice resistant to ischemic AKI, raising the promise of the latter approach in humans. However, trials with anti-ICAM-1 monoclonal antibody administered postischemia did not prevent AKI manifested as delayed graft function in cadaveric transplant recipients [53]. Similarly, initial studies of animals with gene knockouts and treatment with monoclonal antibodies or pharmacologic inhibitor have suggested a role for endothelial E- and P-selectins in the microvascular injury [61–63]. Subsequent studies, though, demonstrated that platelet P-selectin, not endothelial P-selectin, is the key contributor to AKI [64]. Proposed mechanisms include initial adhesion of platelets to the endothelium with subsequent recruitment of leukocytes, or adhesion of platelets to neutrophils with consequent intraluminal cell aggregate formation and trapping in narrow peritubular capillaries [61]. Furthermore, derangements in the coagulation cascade, such as alterations in tissue-type plasminogen activator and plasminogen activator inhibitor-1 in the kidney [65], may combine with the alterations in adhesion molecules to cause the fibrin deposits characteristically found in the renal microvasculature following ischemic injury.

Taken together, while the early alterations in renal perfusion may result from physiological factors regulating vascular tone, sustained impairment of perfusion is likely the result of congestion of erythrocytes and leukocytes in the low pressure, post-glomerular vasculature, leading to the extension phase of AKI (see Fig. 2). These latter events are the likely reason for the lack of efficacy of vasodilator therapy once AKI is established [4, 19, 57, 66]. Pertinent to this issue is the recent finding that retrograde flushing of saline through the renal vein with elevated hydrostatic pressure in the postglomerular vasculature could dislodge the vascular congestion, improve perfusion and inflammation, and hasten recovery of function in rats with established AKI [66].

These observations suggest that targeting microvascular events and inflammation rather that macrovascular hemodynamic events may hold promise in treating established AKI. Despite the lack of evident benefit from the trial of anti-ICAM-1 in limiting AKI in transplant patients, new approaches in the past several years suggest that targeting the microvascular endothelium could have benefit in the setting of AKI. Examples of potential agents might include the use of pro-angiogenic agents such as erythropoietin, vascular endothelial cell growth factor or cell-based therapies using myeloid-derived angiogenic cells, or endothelial colony-forming cells [67].

Nephronal Factors

The term “nephronal factors” refers to the unique susceptibilities of specific nephron segments to injury. Best studied following ischemia are the S_3 segment of the proximal tubule (PT) and the medullary thick ascending limb (mTAL). There has been controversy over the relative importance of each of these segments in AKI [68]. What is clear, though, is that studies of cellular injury and repair in different segments of the tubule have provided important insights into the pathophysiology of tubule cell injury.

Tubule Segment Susceptibility to Injury

Medullary Thick Ascending Limb: The mTAL appears to be particularly vulnerable to hypoxia because oxygen tension in the medulla is low and the mTAL segment has high oxygen consumption [69]. This relationship was defined in isolated kidneys that were perfused with cell-free perfusate. Injury to the mTAL is prevented by increased oxygen delivery through addition of red blood cells to the perfusate. Furthermore, the degree of mTAL necrosis depended on the work load imposed on this segment. Decreasing the work load by inhibiting mTAL solute transport diminished the severity of injury; increasing the work load had the opposite effect. The relationship of these observations to the pathogenesis of AKI in man is controversial, since reducing oxygenation in experimental animals does not reproduce the lesions seen in the isolated perfused kidney model. Nevertheless, studies of the unique vulnerability of this nephron segment to hypoxia have provided insight into the relationship between oxygen supply, work load, and the cellular targets of ischemic, hypoxic, or anoxic injury [68].

Straight Segment of the Proximal Tubule: The straight segment of the proximal tubule (S_3 segment) is highly dependent on oxidative phosphorylation to supply the energy necessary for its bulk transport functions. It is particularly susceptible to ischemia and to nephrotoxins that disrupt energy supply or mitochondrial function [70, 71]. A recent study examined more precisely the long-standing question of tubule segment–specific differences in susceptibility to injury from ischemia. Using a novel model to examine single renal cell ATP levels in vivo during and following ischemia, it was found that ATP levels fell rapidly in PTs and more slowly in distal tubules (DTs). Further confirming earlier reports (detailed in the section following), ATP recovery in PTs depended on duration of ischemia. ATP recovery in DTs was rapid for all intervals of ischemia. In addition, the observed differences in ATP dynamics during ischemia and reflow correlated with differences in cell morphological changes, with PTs being more severely affected than DTs [72].

The earliest alterations in the S_3 segment, after mild or brief ischemia, are found in the brush border and consist of blebbing and internalization of the luminal membrane. With more prolonged ischemia, these changes progress to a more severe form of sublethal injury characterized by cell swelling, mitochondrial condensation, and broader disruption of cellular morphology. Simultaneous with these changes in sublethally injured cells, other cells in this segment may progress to a lethal injury such that the tubule epithelium is denuded from the basement membrane (see Fig. 1). These changes in the S_3 segment result in two important pathophysiologic events, intratubular obstruction and backleak.

Intratubular Obstruction and Backleak

Cell and brush-border debris become impacted in the hairpin turn of the loop of Henle causing obstruction and backleak of tubular fluid in some nephron segments. Intratubular obstruction following ischemic injury has been demonstrated by histomorphology and by finding elevated proximal tubular pressure [71, 73, 74]. The backleak of tubular fluid resulting from loss of tubular integrity in AKI has been demonstrated in both experimental animals and patients [71, 73–75]. In animals, microinjection of inulin or horseradish peroxidase into normal nephrons results in inulin being collected only from that kidney and staining of horseradish peroxidase only in that tubule lumen. In contrast, after microinjection into an injured nephron inulin is found to be excreted by the contralateral kidney and horseradish peroxidase is found in both intra- and peritubular loci. In patients with AKI, backleak has been demonstrated by the differential clearance of graded dextrans [75]. Since filtration is preserved and inulin, urea, and creatinine leak back into the circulation, the renal clearances of these solutes no longer represent GFR in patients with AKI. They nevertheless retain clinical utility in the global assessment of renal clearance ability.

The cellular and molecular mechanisms of tubule obstruction and backleak have been nicely defined [76, 77]. Integrins are outer membrane proteins, basolaterally located in epithelial cells, that attach the cell to the extracellular matrix. Following ischemia or ATP depletion, these adhesion molecules become detached from the extracellular matrix, allowing both dying and viable cells to leave the tubule basement membrane and float into the tubule lumen. In addition, these integrins become displaced from the basal to the luminal cell membrane domain, which allows intraluminal sloughed cells to attach to cells that remain in place, thus contributing to tubule obstruction (Fig. 1). The integrin attachment occurs via an arginine-glycine-aspartic acid (RGD) sequence [76]. The infusion of RGD peptide abolished the characteristic increase in proximal tubule pressure and substantially improved renal function following ischemia, thus confirming the central role of integrin disruption to the processes of intratubular obstruction and backleak in AKI [78, 79].

Cellular and Metabolic Alterations

Adenine Nucleotide Metabolism

Energy depletion and restoration play a pivotal role in renal cell injury [5]. In vivo, renal ischemia causes a profound fall in ATP; in vitro, metabolic inhibition of ATP production is used to induce cellular injury similar to the in vivo ischemia model. Within 5 to 10 min of inducing ischemia, nearly 90% of renal ATP has been consumed [26]. With reperfusion, renal ATP levels recover in a bimodal fashion (Fig. 3). In the first few minutes of reperfusion, there is rapid but incomplete recovery of ATP to 50–70% of normal levels, depending on the duration of ischemia [80, 81]. ATP recovery during the initial phase correlates highly with the total pool of adenine nucleotides (ATP + ADP + AMP) present in the kidney at the end of the ischemic interval. The total adenine nucleotide pool, mainly AMP, decreases as the duration of ischemia increases. So, ATP recovery during initial reperfusion is accomplished primarily by oxidative phosphorylation of the residual cellular nucleotides (AMP and ADP).

A second, slow phase of ATP recovery occurs over hours following the initial rapid phase. ATP recovery rate in the second phase also is a function of the preceding duration of ischemia; longer ischemic intervals result in slower recovery

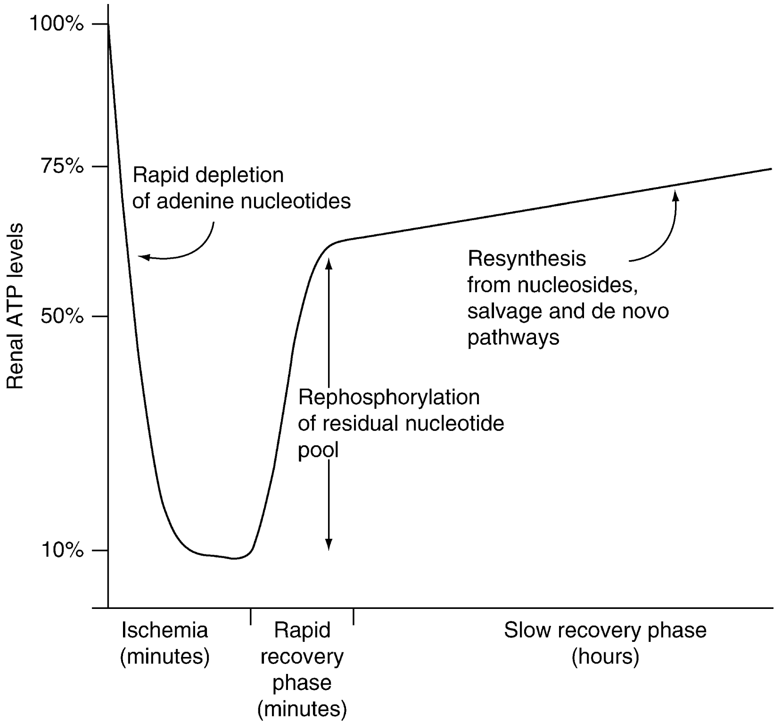

Fig. 3 *Renal ATP depletion and recovery during ischemia and reperfusion*. The initial rapid phase of ATP recovery occurs via phosphorylation of residual renal AMP and ADP. Slow phase recovery represents resynthesis and phosphorylation of adenine nucleotides

rates. The second phase requires resynthesis of ATP from purine nucleotide degradation products and salvage pathways, or from precursors provided during reperfusion (Fig. 3). A primary role for ATP depletion in ischemia-induced epithelial cell injury is supported by several findings: (1) When ATP catabolism is inhibited during renal ischemia, the structural and functional manifestations of AKI are significantly ameliorated [5, 71, 82]; (2) graded reductions in cellular ATP, either in vivo or in vitro, determine the severity of cellular disruption [83, 84]; and (3) augmentation of ATP recovery during reperfusion substantially enhances recovery of renal structure and function [5].

Restoration of mitochondrial structure and function impacts on the later phase of cellular recovery. Mitochondrial respiratory protein abnormalities persist for 6 days after the insult in two different animal models of AKI, and markers of mitochondrial biogenesis are elevated in both. So disruption of mitochondrial homeostasis persists even in the presence of mitochondrial recovery signals [85]. Administration of a pharmacological activator of mitochondrial biogenesis, after induction of AKI by ischemia, expedited recovery of mitochondrial proteins and function, along with faster recovery of markers of tubule polarization and differentiation [86].

Therefore, impaired adenine nucleotide metabolism and mitochondrial homeostasis are both a consequence and predictor of renal cell injury and recovery. Furthermore, altered ATP metabolism triggers several pathways that lead to deranged epithelial structure and function: reactive oxygen molecules, increased intracellular calcium, activation of phospholipases, and disruption of tubule cell architecture. Each of these downstream events is discussed below.

Reactive Oxygen Molecules

Reactive oxygen molecules (ROM) have been implicated in a variety of kidney diseases, including reperfusion injury after ischemia [87]. The most highly reactive oxygen molecules are free radicals such as hydroxyl radical (OH) or superoxide anion (O_2-). The high reactivity and brief existence of these molecules result in injury close to the site of free radical generation. Other oxygen species such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), not being free radicals, are less reactive. However, they have longer half-lives than free radicals and may cross cell membranes to cause injury distant from their site of origin.

ROM are generated during reperfusion of kidneys [87]. Ischemia induces metabolism of the adenine nucleotides to adenosine, inosine, and hypoxanthine. Ischemia also may cause a conformational change in xanthine dehydrogenase (which uses NAD as an electron receptor) to xanthine oxidase, which uses oxygen [88]. Once perfusion and oxygen delivery are returned, xanthine oxidase can metabolize hypoxanthine to xanthine and uric acid, generating hydrogen peroxide and superoxide anion. Mitochondria, which produces reactive species though electron transport systems, when damaged by ischemia may leak the injurious oxygen metabolites. The respiratory burst and myeloperoxidase released from activated PMNs produce superoxide anion, hydrogen peroxide, and hypochlorous acid [87]. Prostaglandin metabolism may also provide reactive molecules. Iron can play a significant part by donating electrons to hydrogen peroxide to form the highly damaging hydroxyl radical. In this way, iron release may be responsible for the injury resulting in hemoglobin- or myoglobin-induced AKI [89].

Once generated, ROM damage cellular components and extracellular matrix [5]. Intracellular proteins can be oxidized, resulting in conformational changes with loss of enzymatic activity or compromised integrity of structural proteins. Membrane lipids and lipoproteins undergo peroxidation by free radicals; these propagate and result in progressive membrane damage. Hydroxyl radicals damage DNA and oxidize matrix proteins. Reactive oxygen metabolites may contribute to ischemia by deleterious interactions with nitric oxide synthetases or with NO itself. NO can combine with superoxide to form peroxynitrate, a potent oxidant.

Because of these putative actions, much attention was focused on ROM in ischemia-reperfusion injury [19, 80, 87]. Animal models show ROM involved in the pathogenesis of AKI, but whether ROM play a significant role in human AKI has long been questioned. The effects of enhancing endogenous antioxidants (superoxide dismutase, glutathione, catalase, apotransferrin, or neutrophil gelatinase-associated lipocalin, NGAL) or administering exogenous free radical scavengers (*N*-acetylcysteine, deferoxamine, or edaravone) have been examined. Animal studies have shown benefit from treatment with the antioxidants or free radical scavengers, but similar translational studies in humans, so far, have been inconclusive or have shown no benefit.

However, there was found a significant increase in oxidative stress in humans with AKI [90]. In addition, genomic studies identified an association between serum catalase and a specific catalase allele with the severity of AKI in ICU patients [91]. So, despite the large number of studies on this topic, no final conclusion can be made as to the importance of reactive oxygen species in the pathogenesis of AKI, since strong evidence exists both for and against these compounds having a dominant role.

Intracellular Calcium

Under normal circumstances, extracellular fluid calcium concentration exceeds that of intracellular free calcium (Ca_i) by a factor of 10^4. The remarkably low Ca_i is maintained by a variety of mechanisms that remove calcium from the cell or sequester it within intracellular compartments. Calcium is extruded from cells by plasma membrane Ca-ATPase and the Na/Ca-exchanger. Intracellular sequestration is mediated by Ca-ATPase in the endoplasmic reticulum and by calcium uptake into mitochondria. A fall in cellular ATP would be expected to reduce the activity not only of Ca-ATPase, but also Na/K-ATPase and thereby affect the Na/Ca-transporter. The net effect of cellular ATP depletion on extrusion of Ca from the cell and sequestration in the endoplasmic reticulum, then, would be to decrease calcium transport contributing to a rise in Ca_i. In addition, it has been suggested that release of Ca from cellular organelles contributes substantially to the rise in cytosolic Ca.

The contribution of mitochondrial damage to the observed rise in cytosolic calcium after ischemic or hypoxic injury appears to be minimal, because even damaged mitochondria are able to sequester calcium actively [80]. However, the converse effect may be a mechanism in lethal cell injury. That is, the increase in cytosolic Ca that occurs through mechanisms outlined above can lead to unrestricted uptake by mitochondria. A substantial rise in mitochondrial calcium levels, then, occurs after cell injury is lethal.

The question whether high Ca_i levels play a central role in the pathophysiology of cell injury or whether Ca_i changes are merely secondary to cell death has been controversial. Increased cell and mitochondrial Ca levels are linked to cell death, but were thought not to increase until irreversible changes had occurred [92, 93]. Later studies, however, demonstrated a reversible increase in Ca_i early in the course of cell injury in isolated proximal tubules [94]. In an in vitro model, it was found that graded levels of ATP depletion caused significant, early increases in Ca_i in renal cells, but did not cause cell death. Furthermore, the increases in Ca_i were tightly and inversely correlated to the level of ATP depletion, and preceded disruption of membrane protein-cytoskeleton interactions and induction of putative recovery mechanisms through the stress response [84]. Finally, attenuation of ischemia-induced AKI is found in mice deficient in the Na/Ca exchanger and in animals administered compounds that inhibit this exchanger, both models that would be expected to diminish the secondary rise in Ca_i mediated via the reverse mode of the Na/Ca exchanger during and after ischemia [95, 96]. Therefore, alterations in Ca_i appear not to be merely a terminal manifestation of lethally injured cells, but play an important early role in the pathogenesis of ischemia-induced AKI.

Increased Ca_i may play an early role in AKI by mediating several aspects of sublethal injury, and a later role in a subsequent transition to lethal injury after a more severe insult. An increase in Ca_i may activate calpain, contributing to degradation of the cytoskeleton; it could increase activity of other proteases and phospholipases that contribute to either sublethal cell disruption or cell death; and it could inhibit mitochondrial oxidative phosphorylation. The latter effect would lead to a vicious cycle of progressive cell energy depletion, further cell injury, and eventual cell death. However, alterations in Ca_i also could activate recovery mechanisms, such as induction of the stress response [84]. Furthermore, increased cytosolic calcium dramatically induces calcium-binding proteins such as annexin A2 and S100A6, which play an important role in cell proliferation during recovery from AKI in animal models [97]. The relative contribution that change in Ca_i makes to specific pathophysiological versus recovery mechanisms in AKI is yet to be defined.

Phospholipids and Lipases

The striking morphological changes that occur in proximal tubules after ischemia, especially brush border membrane blebbing and the release of intracellular enzymes such as lactate dehydrogenase, suggest that significant membrane alterations are part of the cellular injury in AKI. Changes in intracellular calcium flux activate endogenous phospholipase, and some phospholipase may be activated by decreased cellular ATP. Phospholipase activation causes breakdown of membrane phospholipids into free fatty acids and lysophospholipids, which can disrupt membranes and be precursors of active metabolites that promote inflammation and further activate phospholipases. Moreover, the lack of ATP during ischemia may prevent the synthesis of new phospholipids to replenish membranes. The result, then, is a generalized loss of membrane phospholipids with an accumulation of degradative products that by their detergent activities can further disrupt cell membranes, eventually leading to loss of membrane integrity and cell death (reviewed in detail in ref [98]).

As discussed earlier, another mechanism that may contribute significantly to progressive membrane deterioration is injury by oxygen free radicals. ROM generated during reperfusion peroxidate lipids, which can propagate through other membrane lipids causing widespread membrane damage. In fact, signs of lipid peroxidation provide significant evidence for free radical generation during reperfusion. It is suggested that certain phospholipase isoforms may limit, rather than enhance, membrane injury by hydrolyzing phospholipids oxidized by ROM [98]. Removal of the damaged, oxidized lipids are thereby facilitated, limiting progressive membrane damage from these toxic metabolites.

Renal ischemia causes activation of phospholipase (PLA_2) and a rapid fall in cortical phospholipid levels [99]. The detrimental effects of PLA_2 can be offset by unsaturated free fatty acids [100], suggesting that PLA_2 may be cytotoxic by degradation of phospholipids in cell membranes or through accumulation of lysophospholipids. It appears, though, that the role PLA_2 plays in renal cell injury depends on both the particular isoform of PLA_2 as well as the insult applied to cause AKI [98]. For example, cytosolic calcium-dependent PLA_2 is implicated in mediating oxidant-induced renal cell injury. On the other hand, endoplasmic reticulum Ca-independent PLA_2 appears to have a role in phospholipid repair in proximal tubules that varies according to the insult applied [101].

Just as phospholipase activity may vary according to the stimulus and domain, and either contribute to or limit the injury depending on the context, the role of lipid alterations similarly can differ in AKI. While free fatty acid and lysophospholipid accumulation may contribute to membrane damage, cholesterol accumulation appears to stabilize plasma membranes and limit renal cell injury in models of AKI. Acute tubule injury initially lowers, then increases, cholesterol content in renal tubule cells [102]. When renal cells were treated with a variety of agents to reduce cholesterol content, cell susceptibility to injury from ATP depletion was substantially increased, suggesting that cholesterol is a cytoprotectant in the context of ischemic AKI [103].

So, while the understanding of lipid alterations in AKI has become more complex, the story is similar to that of changes in Ca_i. The role of these alterations may be pathologic or adaptive, depending on the type and severity of the insult, on the location of the intracellular perturbations, and on the particular isoforms active in the affected areas.

Disruption of Tubule Cell Architecture

Renal tubule cells are highly specialized for their primary function of ion and small molecule transport, which depends on their polar structure. The change in proximal tubule cell polarity that occurs during AKI has profound effect on this function (Fig. 4) [104]. The plasma membrane of proximal tubule cells is divided into apical and basolateral domains by tight junctions shared with neighboring cells. The apical membrane, facing the tubule lumen, differs from the basolateral membrane both in lipid and membrane protein composition. The microvilli of the apical domain are rich in sodium-dependent cotransporters and Na/H-antiporters, which effect reabsorption of glucose, amino acids, phosphate, and bicarbonate along with sodium. The sodium gradient across the apical membrane, which drives the function of these transporters, is established and maintained by Na/K-ATPase located on the basolateral membrane. Plasma membrane polarity is maintained by the tight junctions that prevent lateral diffusion of membrane phospholipids and proteins into the opposite domain. In addition, the cortical cytoskeleton anchors membrane proteins, such as Na/K-ATPase, in specific domains through an intricate sequence of linked proteins. The actin-based cytoskeleton is a dynamic structure that maintains cellular polarity and mediates a number of processes necessary to sustain both structure and function of renal epithelia [105]. Interactions between cytoskeletal proteins and plasma membrane proteins are responsible for functions that include cell adhesion, endocytosis, signal transduction, and activity of ion channels.

The loss of cellular polarity is a fundamental alteration in renal epithelial injury that causes reduced transepithelial sodium reabsorption. Renal ischemia disrupts normal renal cell polarity. Proximal tubule tight junction integrity, an obstacle to diffusion of membrane components across domains in healthy tubule cells, is lost. Membrane lipids and proteins that are highly mobile

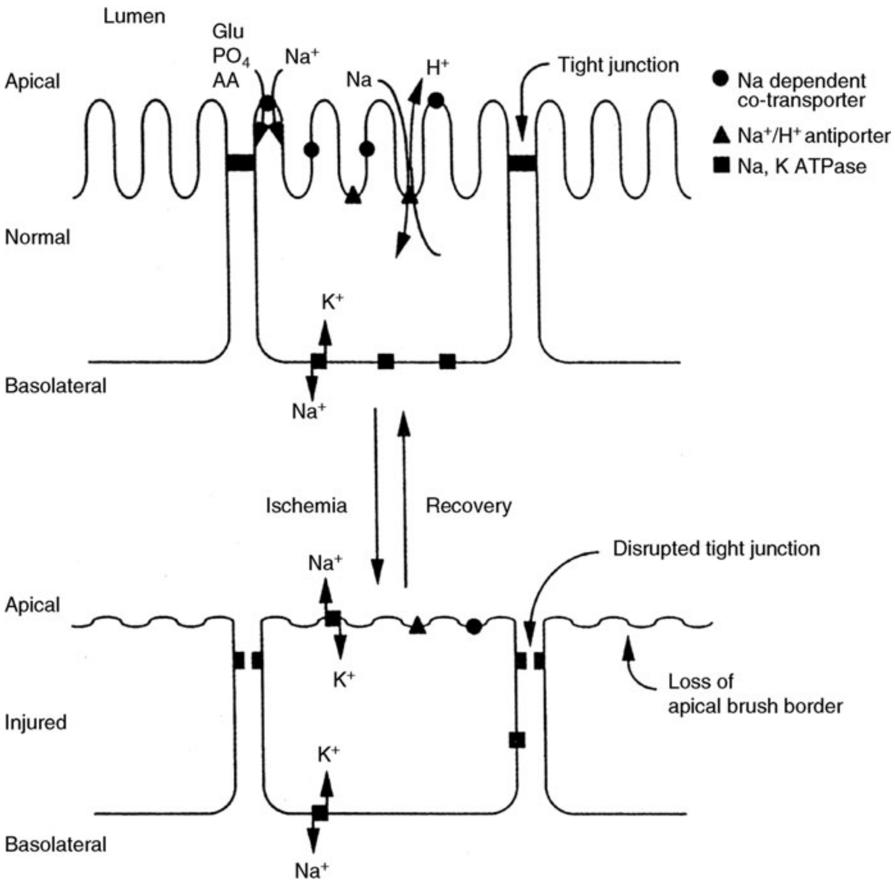
Lumen
Apical
Glu
PO_4
AA
Na^+
Na
H^+
Tight junction
● Na dependent co-transporter
▲ Na^+/H^+ antiporter
■ Na, K ATPase
Normal
K^+
Basolateral
Na^+
Ischemia
Recovery
Disrupted tight junction
Na^+
Apical
K^+
Loss of apical brush border
Injured
K^+
Basolateral
Na^+

Fig. 4 *Disruption of proximal tubule cell polarity.* Ischemia causes loss of apical brush border, opening of tight junctions, and loss of cell polarity that impairs transepithelial solute reabsorption. Recovery of function parallels recovery of cell architecture

redistribute between apical and basolateral domains. Membrane proteins such as Na/K-ATPase, which normally are anchored to the actin cortical cytoskeleton, can migrate when this attachment is broken (Fig. 4). After as little as 5 min of renal ischemia there is loss and internalization of brush border membranes, and basolateral Na/K-ATPase migrates to apical membranes [104].

The actin cytoskeleton plays an important role in this process. Renal ischemia alters actin microfilaments and organized polymerization in proximal tubule cells [105, 106]. Actin disruption itself results in opening of tight junctions and loss of the polar distribution of those proteins, such as spectrin, that anchor Na/K-ATPase to the plasma membrane [81, 107–109]. Actin depolymerizing factor (ADF, also known as cofilin) participates in ischemia-induced actin cytoskeletal alterations and determines the rate and extent of these ATP depletion-induced changes in actin. ADF/cofilin is normally maintained in an inactive form by Rho GTPases. ATP depletion inactivates Rho GTPase, causing activation and relocalization of ADF/cofilin to the surface membrane [110, 111]. Concomitantly, ATP depletion dissociates the actin stabilizing proteins tropomyosin and ezrin, allowing activated ADF/cofilin to bind and cleave actin, leading to microvillar breakdown [112]. Finally, atypical protein kinase (aPKC) signaling, impaired during ATP depletion, participates in tight junction disassembly during cell injury. Furthermore, aPKC function is important for tight junction reassembly during recovery, a feature necessary for restoration of cell polarity and tubule integrity [113].

The ischemia-induced alteration of renal tubule cell architecture, then, underlies the reduction in transepithelial sodium reabsorption, which is manifested clinically as increased fractional excretion of sodium and is a hallmark of AKI. Recovery of normal sodium reabsorption occurs after normal renal cell structure and membrane polarity, in sublethally injured cells, is restored during recovery from AKI.

Cell Death

Necrosis and Apoptosis

After an insult cells can proceed down one of several pathways: recovery from sublethal injury or death by necrosis or apoptosis. Individual cells can simultaneously be moving through each of these pathways in AKI. The hierarchy of injury that results depends on the cell type, location, and severity of the insult [84, 114, 115]. Cell death by necrosis tends to follow a severe insult that leads to metabolic collapse of the cell and is more prominent in the outer medulla where the severity of the injury is exacerbated by vasoconstriction and microvascular congestion. Necrosis is massive and rapid leading to cell lysis with secondary injury to surrounding tissue. Typical features of necrosis include cytoplasmic swelling, loss of membrane integrity, and nuclear and cellular fragmentation. Cell death from apoptosis is a more regulated process that leads to cytoplasmic and nuclear shrinkage, DNA condensation and fragmentation, and eventual cell breakdown into membrane-bound apoptotic bodies. These apoptotic bodies in vivo are cleared rapidly by other cells, which has made the assessment of how much apoptosis occurs following a renal injury difficult.

While clinical acute renal failure from ischemic or toxic injury has long been called acute tubular necrosis (ATN), this term has been questioned since biopsy of patients with ARF rarely show necrotic features. However, kidney biopsies typically are late during ARF and may miss earlier signs of necrosis. Animal models of ischemic or toxic injury clearly show at least focal areas of necrosis early during reflow. Current understanding that a renal insult can lead to simultaneous sublethal and lethal injury (necrosis or apoptosis) in cells in different areas of the kidney has led to the change in terminology from ARF or ATN to acute kidney injury (AKI, see introduction) [1].

Measuring apoptotic cell death in vivo has been problematic, leading to controversy over whether this is an important pathway in AKI. Definitive morphologic criteria have established and confirmed apoptosis as a pathway to cell death in renal epithelia both in vivo and in vitro [116, 117]. Biochemical assays can be helpful to quantify apoptosis, but are not uniformly reliable in vivo or in vitro, unless traditional cell morphology criteria are also applied. For example, neither endonuclease activation nor DNA laddering consistently discriminate between necrosis and apoptosis in injured renal epithelia [118, 119]. However, the specificity of TUNEL staining for apoptosis in several forms of in vivo renal cell injury was determined using accepted morphological criteria and was found to be 99% specific in vivo ischemic renal injury [120]. These studies indicate that apoptosis is an important mechanism of cell death in AKI leading to exploration of apoptotic pathways that might be active after a renal injury.

The process of apoptosis has been separated into three sequential phases: initiation, commitment, and execution phases. The initiation phase can be activated by extrinsic factors or intrinsic cell stress [19, 121]. Membrane receptors and the Fas pathway can contribute to the extrinsic initiation phase of apoptosis in ischemia, and interference with this pathway can ameliorate in vivo AKI [122–125]. Intracellular derangements in cell architecture, metabolism, and function during ischemia are likely a major trigger to the intrinsic pathway for initiation of apoptosis following ischemia.

Central to the commitment phase of apoptosis are several changes in mitochondria. Loss of mitochondrial transmembrane potential is pivotal to the cascade; interventions preventing mitochondrial permeability transition avert apoptotic nuclear and cell membrane alterations [126–129]. Bax translocation from its cytoplasmic pool to mitochondria contributes to opening of mitochondrial pores with subsequent release of cytochrome C [130–132].

Bax-mediated apoptosis has been demonstrated in cultured proximal tubule cells following ATP depletion [132]. An imbalance between the pro-apoptotic (Bax, Bid) and antiapoptotic (Bcl-2, Bcl-xL) members of the Bcl-2 protein family, as well as the proapoptotic protein p53, are implicated in the commitment phase of apoptosis in both animal [133–135] and human [136–138] conditions of ischemia-induced AKI. Though cytochrome C release from mitochondria is not specific for apoptosis, it follows the mitochondrial permeability transition that is modulated by the Bcl-2 protein family and indicates commitment to cell death. Its release is an essential factor in causing downstream activation of the effector proteins for apoptosis [115, 126, 132].

Caspase 3 is the effector protein that lies at a critical juncture in apoptosis pathways. Activation of caspase 3 begins the execution phase of apoptosis [139–141]. A series of additional caspases are activated in the execution phase that cleave multiple cellular proteins, including cytoskeletal-associated proteins and endonuclease inhibitors, resulting in the characteristic morphology of apoptosis – cell shrinkage, membrane blebbing, nuclear condensation, and fragmentation [115].

Autophagy

Autophagy is a cell stress response involving bulk degradation of damaged organelles, protein aggregates, and macromolecules in cytoplasm. This cellular mechanism is suggested to be an adaptive response that protects against AKI by limiting cell death. Indeed, studies indicate that blocking autophagy pharmacologically or genetically accelerates apoptosis [142]. As such, autophagy may be a pathway to be manipulated in preventing AKI.

Inflammatory Response

Although studies many years ago suggested that the immune system had no role at all in ischemia-induced AKI, a growing body of evidence in the past several years indicates that the inflammatory response plays a major role in AKI [53, 143, 144]. The inflammatory cascade is initiated at the vascular endothelium. Renal injury leads to increase in the expression of leukocyte adhesion molecules ICAM-1 and P- and E-selectin on endothelial cells, and interference in these pathways results in less inflammation and injury after renal ischemia [145–148]. Inflammatory cascades initiated by endothelial dysfunction can be augmented by potent mediators generated by the ischemic proximal tubule. Both pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, and TGF- β) and chemotactic cytokines (MCP-1, IL-8, and RANTES) are generated following renal ischemia and can enhance inflammation [144, 149–153]. Strategies to modulate the inflammatory response by altering cytokine action have been tried in AKI. Modulation of IL-6 and IL-10 have demonstrated initial results to reduce overall inflammatory injury. Signaling of Toll-like receptors (TLR) on immune cells from any stimuli causes an inflammatory state, which when uncontrolled can lead to tissue damage [154–157]. The common mediators of signaling from the TLR family include TLR-2 and TLR-4. Inhibition of TLR-2 by gene knockout or antisense treatment prevents ischemia-induced renal dysfunction and suppresses some cytokines in cultured proximal tubule cells [157, 158]. Conflicting findings of studies on TLR pathways in AKI indicate not only that the effect may vary according to the particular renal insult, but that TLR pathways need to be explored in more detail to define clearly their role in AKI.

Cellular and chemical signal disruption may be prominent in inflammation associated with AKI. Cellular attraction and chemoattraction of cytokines and chemokines by injured kidneys have been documented following ischemia [143, 146, 153, 159–164]. The significance of neutrophil infiltration is not clear because neutrophil depletion or blockade of function provides only partial protection and only in some animal models, and neutrophil infiltration appears not to be a prominent feature of ischemic renal injury in humans. Selective macrophage depletion and manipulation of macrophage infiltration into the kidney decreases the severity of AKI [165–167]. Macrophages are dependent on coordinated action of T cells and neutrophils. Although it is the most abundant immune cell in the kidney, the role of the dendritic cell in AKI is not known.

T and B cell determinants of inflammation are also contributory in AKI. The role of T cells has been intensely studied; they have been found in the kidney in both human and rodent ischemic AKI [144, 153, 168]. Reversal of protection in the latter model occurs with adoptive transfer of wild-type T cells into the knockout mice. T regulatory cells were determined to have a fundamental role in balancing the inflammatory response within the kidney in AKI [169–171]. Resistance to developing AKI following preconditioning is attributed to T regulatory cells. Along with numerous agents that promote proliferation, function, and trafficking of endogenous T regulatory cells, studies using adoptive transfer of T regulatory cells indicate their protective role in AKI [172]. Compared with wild-type animals, B-cell-deficient mice kidneys, with comparable T cell and neutrophil infiltration following ischemic renal injury, are partially protected both structurally and functionally [146, 148, 164, 172–174].

Ischemia-reperfusion injury in most organs activates the classical pathway of the complement cascade. However, the alternative pathway, along with the mannose-binding lectin pathway of complement activation, appears to predominate in ischemic AKI in animals and humans. Studies focused on C5a, a powerful chemoattractant for inflammatory cells, suggest that it contributes to renal injury and impairs recovery. Another complement protein, factor B, may also have an integral role in augmenting the inflammatory response in ischemic AKI [175–178].

The clear involvement of the immune system in ischemic AKI suggests a mechanism underlying the consistent observation that development of acute kidney injury substantially increases overall morbidity and mortality in patients suffering from a variety of diseases, including isolated cardiac disease, sepsis, and multiorgan dysfunction. In that context, it has been intriguing to see animal studies documenting distant organ effects and dysfunction in the heart, lungs, and brain after isolated ischemic AKI [149, 153, 160, 179–181]. It has been suggested that these distant effects of renal ischemia may be mediated by inflammatory signals generated in the injured kidney.

Sepsis-Induced AKI

Sepsis and acute kidney injury synergistically increase morbidity and mortality in hospitalized patients. Sepsis is the leading condition associated with AKI [182]; the BEST kidney study reported an AKI incidence of 5.7% in 29,000 patients with sepsis being the highest associated etiology (47.5%) [183]. Analysis of 276,731 admissions to 170 adult critical care units in the UK identified concurrent sepsis and AKI in 8246 ICU admissions in the first 24 h [184]. Sepsis-associated AKI (S-AKI) is common in children, particularly in the developed nations. Though separate entities, sepsis and AKI carry considerable pathophysiologic overlap and significantly worsen patient outcomes when concurrent. Sepsis is the condition most associated with AKI in critically ill patients, so significant effort has been expended to understand the pathophysiologic drivers of each separately and together. Despite abundant epidemiologic data describing incidence and associated outcomes, understanding of the complicated pathophysiology driving S-AKI is only superficial, appreciation of the disease phenotype is lacking, and current diagnostics are outdated. Ultimately, these shortcomings contribute to a deep knowledge deficit, resulting in a paucity of focused and effective therapeutic options.

The pathophysiology of S-AKI is multidimensional and occurs on the microscopic and macroscopic level [185–189]. A combination of subcellular inflammatory dysregulation, cellular end events ranging from apoptosis to necrosis to quiescence, endocrine and paracrine oxygen delivery (DO_2) dysregulation and dysoxia at the end-organ level, and aberrancy in blood flow and perfusion are all believed to be mediators of S-AKI. Both murine and ovine models have been used to delineate the drivers of injury in S-AKI including: direct endotoxin administration using lipopolysaccharide (LPS) through intraperitoneal or intravenous injection, direct live bacterial instillation (either gram negative or gram positive), cecal ligation and puncture (CLP) with fecal implantation, and surgical bacterial implantation [185, 186, 190]. In addition to the pro-inflammatory and chemoattractant signals discussed in the previous section, the importance of the pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) have been identified in relation to pathogen recognition receptors such as TLRs [191–194]. DAMPs and PAMPs directly and indirectly induce renal tubular damage and contribute to loss of function. Oxidative stress is associated with renal tubular damage. In LPS models, endocytosis by S1 tubular epithelial cells triggers an oxidative burst in the S2 tubular segment cells, potentially secondary to suppression of heme-oxygenase 1 and Sirt1, both being protective against oxidative damage [195–198]. Oxidative stress then affects the balance of cell survival, arrest, necrosis, or apoptosis. Proapoptosis gene regulation is notably increased in models of S-AKI, particularly by altering GSK3β function, which is known to balance TNF-α and other regulators of apoptosis. Endothelial activation is not only triggered by the interleukin inflammatory cascade but also modulated by TNF-α mediated caspase-8, identified as a mediator of AKI in sepsis but not in ischemia. Other apoptotic mediators such as p53, p21, bax, and cyclin-dependent kinase 2 are also implicated, identified by therapeutic interventions such as zVad pancaspase inhibitor, PI3k/Akt antiapoptotic pathway activators, and JNK inhibitors in animal models [165, 174, 181, 199–201].

A significant point of interest is the role of the dysregulated inflammatory system in S-AKI and whether this is distinct from ischemic AKI. Comparison of gene dysregulation in the separate models of AKI help elucidate the differences. In CLP models of AKI, compared with ischemic models of AKI, NF-kb/MYD-88 signaling of the pro-inflammatory transcriptome appears to contribute significantly to the inflammatory process in the kidney, outside of independent renal production of these gene transcripts. Sepsis itself appears to modulate TLR-4 responses, as seen in TLR-4 knockout mice subjected to CLP compared with the ischemic AKI model. This raises a fundamental question. Is kidney dysfunction during sepsis an effect of the host response to infection and inflammation (i.e., the kidney is an "innocent bystander") or is the kidney independently responsible for propagating injury and inflammation? This differential paracrine versus autocrine response is poorly understood at this time, but has clear clinical implications for protection or recovery of renal function in sepsis [149, 159, 160, 202].

Histopathology demonstrates wide variability in the phenotype of septic AKI in humans. Although both murine and ovine models indicate that apoptosis is more common than necrosis, the available evidence suggests that neither are common in human S-AKI. Less than 20% of available biopsy results show any morphological aberration in tubular or mesangial cells or distortion of architecture. The animal models of CLP, small or large animal, show that necrosis is not a typical finding in sepsis-associated AKI [186, 203–206].

Differences in renal perfusion in sepsis compared with ischemia may explain the discrepant findings of histopathology, inflammation, and cellular survival in the two forms of AKI [204, 207]. In contrast to ischemic AKI, increased renal blood flow and glomerular perfusion are the norm in ovine models of LPS-induced AKI. Renal blood flow during S-AKI has direct correlation with cardiac output. Sepsis has asymmetric effects on the afferent and efferent renal arterioles, with relatively reduced efferent arteriole resistance in that setting. The net effect is to reduce transglomerular pressure, thereby reducing GFR.

The issue in sepsis, then, appears not to be about oxygen delivery per se, but poor oxygen utilization, or dysoxia. Sepsis-induced disruption of bioenergetics is manifested by reduced glycolytic capacity, reduced glycolytic reserve, and reduced ATP reserve production in CLP models. The role of mitochondria in ischemic AKI has been extensively explored, and the gene targets of the PPAR and PGC-1a are implicated in the bioenergetics failure resulting from pedicle clamping or hypoperfusion models [208–211]. Mitochondria are also common targets of inflammatory injury induced by sepsis, leading to dysfunction and increased production of injurious reactive oxygen species.

Endothelial injury in septic models affects vascular permeability throughout the kidney, including in the glomerulus and peritubular microvasculature, facilitating inflammation with fluid extravasation and propagating organ injury. A related phenomenon is hyperviscosity and sludging in the microvasculature. Microthrombi formation can be seen within 4 h after CLP, thought to be secondary to production of reactive oxygen and nitrogen species.

In sum, then, several concurrent processes contribute to an environment within the kidney to cause sepsis-induced AKI. Among these include the endothelium in the microvasculature and renal tubule epithelia being primed for dysoxia and poor oxygen uptake during sepsis and ischemia. Given the evidence, the scope of injury in S-AKI suggests that the direct dysregulation found in ischemic models of AKI, including immune trafficking by chemoattractants and macrophage-monocyte infiltration, is as detrimental to function as the indirect effects of sepsis-mediated injury.

The renal system can participate in protection from sepsis-triggered AKI mediators. Several animal models have shown induction of mitophagy, autophagosomal capture of dysfunctional mitochondria, during the production of oxidative species in sepsis [212–215]. In addition, cell cycle arrest early in sepsis may be an important defense mechanism against injury to cells in the nephron, thereby helping prevent energetic failure in the affected cells and limiting propagation of injury. To this end, the cell cycle arrest marker TIMP2/IGFBP7 (tissue inhibitor of matrix metalloproteinase 2/insulin-like growth factor binding protein 7) is a validated marker of AKI in septic critically ill patients.

Identification of potential therapeutic targets in S-AKI depends on understanding the individual processes propagating injury (Fig. 5). A unified theory of sepsis and AKI could incorporate the individual cellular and subcellular drivers of injury. These range from the inflammatory danger signal (DAMPs and PAMPs) to the oxidative stress and perturbed oxygen delivery, and to the aberrant renal blood flow and dysregulation of vascular tone (mediated by ICAM and selectins described earlier). However, differences in the insults causing AKI indicate that the balance of oxidative stress and locally acting cytokine

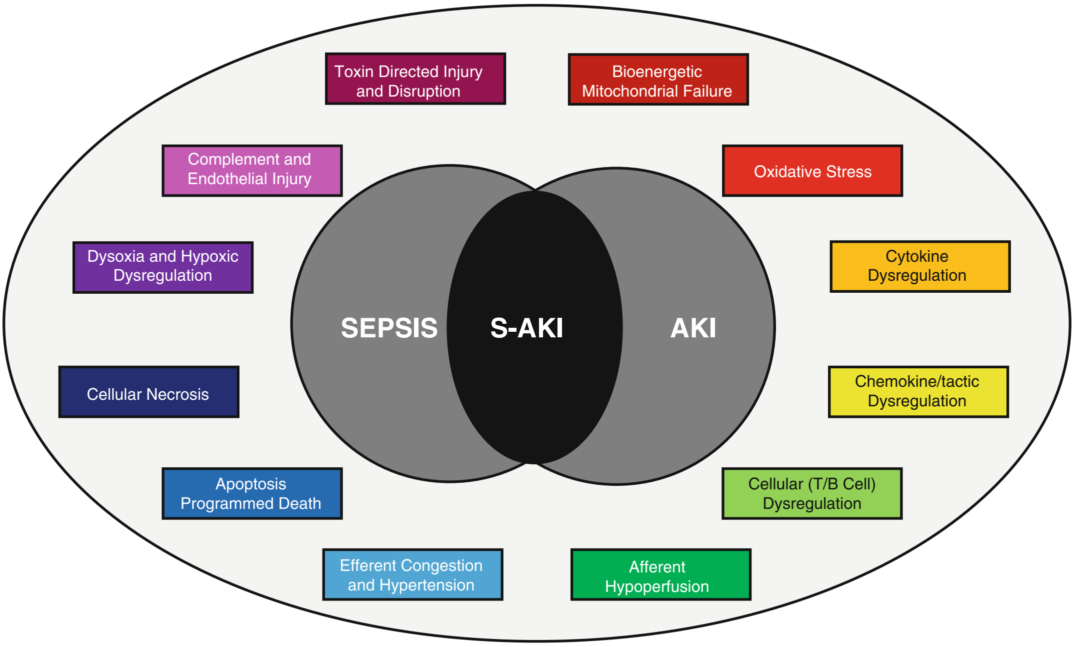
Toxin Directed Injury and Disruption
Bioenergetic Mitochondrial Failure
Complement and Endothelial Injury
Oxidative Stress
Dysoxia and Hypoxic Dysregulation
Cytokine Dysregulation
SEPSIS
S-AKI
AKI
Cellular Necrosis
Chemokine/tactic Dysregulation
Apoptosis Programmed Death
Cellular (T/B Cell) Dysregulation
Efferent Congestion and Hypertension
Afferent Hypoperfusion

Fig. 5 The drivers of sepsis, acute kidney injury (AKI), and sepsis-associated AKI (S-AKI)

mediators appears to induce a state of pro- or antiapoptotic forces in S-AKI, debunking the previously held dogma that equated septic AKI with acute tubular necrosis.

Currently, the animal and in vitro models of sepsis and AKI carry both strengths and limitations. The nonsurgical models remain easier and less expensive but are often not physiologic and vary significantly in reproducibility of findings from in vivo models. In addition, the surgical models of S-AKI suffer from the lack of a precise equivalent in humans. Specifically, CLP is not a mimic for nonabdominal sepsis. Nevertheless, these models have substantially advanced our understanding of the pathophysiology of sepsis as a major cause of AKI, and how mechanisms triggered by sepsis coincide with or differ from ischemia-induced AKI. Moving forward, continuing to define precisely biological markers of cellular injury may facilitate their clinical implementation in various forms of AKI. In so doing, well-defined biomarkers could improve prognostication, and further help untangle the complex and unique biology of sepsis-associated AKI from both sepsis alone and ischemia-induced AKI.

Genetic Susceptibility

In the clinical realm, it has long been observed that the incidence or severity of AKI varies widely between patients who have apparently been subjected to the same renal insult. While unrecognized environmental factors certainly play a role, this observation has led clinicians to suspect that individual patients may have an inherent genetic susceptibility or resistance to developing AKI. This has led investigators to explore whether differences in genetic background affect the risk of developing AKI.

Specific human genetic polymorphisms have been linked to either resistance or susceptibility to develop AKI. These human studies have implicated genetic polymorphisms of pro- and anti-inflammatory cytokines and heme oxygenase-1 (HO-1) as potential determinants of susceptibility to ischemic renal injury [216]. Genetic polymorphisms of the stress protein Hsp72 were associated with neonatal susceptibility to develop AKI. The Hsp72 (1267) GG allele has low inducibility of the Hsp72 protein. Neonates carrying the Hsp72 (1267) GG genetic variation had increased risk of AKI [217, 218]. Furthermore, several maternal and fetal cytokine genetic polymorphisms were linked to the incidence and severity of sepsis, necrotizing enterocolitis, and eventual AKI [218].

The proliferation of such studies led to a systematic review to determine whether any polymorphism could be conclusively associated with AKI. Of 35 individual polymorphisms whose association with AKI had been reported and examined in the review, only 1 polymorphism, *APO E e2/e3/e4* (a regulator of inflammation), had more than one study showing a significant impact on AKI incidence [219]. However, that association was not confirmed in a larger study. The authors concluded that since current association studies do not provide definitive evidence linking specific genetic variations to AKI, a different approach is required. They conclude the need to restrict such studies to a narrow consensus definition of AKI (as discussed in the introduction to this chapter), and a shift from a priori hypothesis-driven to genome-wide association studies [219].

Studies of different rat strains give credence to this approach, along with the concept that genetic background can substantially influence vulnerability to AKI. The inbred Brown-Norway (BN) rat strain is profoundly resistant to developing AKI following ischemia when compared with other inbred and outbred rat strains [220]. The strain specific protection appears to be on a cellular level since immediate cellular manifestations of ischemic AKI are limited, and the protection is sustained. The specific mechanism providing this protection is yet to be determined, but that it is related to differential gene expression is indicated by the finding of increased constitutive expression of putative cytoprotectants in the protected strain.

To define the genetic basis for resistance to AKI further, consomic rats were studied, in which individual chromosomes from the AKI-resistant BN rat were placed into the genetic background of an AKI-sensitive rat strain. Nine chromosomes on consomic strains were found to contain alleles that afford protection against developing AKI [221]. So, full protection against ischemic injury in the BN rat likely is from multiple beneficial alleles working in concert. In silico analysis identified at least 36 candidate genes on the chromosomes found to contain protective alleles, with several candidate genes previously linked to the pathophysiology of AKI [221]. So natural variants of these alleles, or yet to be identified alleles on the chromosomes identified to harbor protective genes, provide protection against AKI in the rat. Since both the rat and human genomes have been fully sequenced, the results from this study might guide translational studies in humans to identify modulators of AKI in susceptible patients. This might then allow identification of individuals genetically predisposed to develop AKI in a specific clinical setting.

Biomarkers

Advances in functional genomics and cDNA microarray-based technologies have revealed the landscape of the numerous pathways activated by acute kidney injury [222–226]. Novel genes with altered expression and new signal transduction pathways have been discovered that may serve as biomarkers of AKI. The ectodomain of kidney injury molecule-1 (KIM-1) protein, upregulated following ischemia on the apical membranes of proximal tubule cells, is cleaved and released into tubule lumen. This sliced part of KIM-1 is detected early in urine even with mild proximal tubule injury from a variety of causes. Increase in urinary KIM-1 has been shown to be prognostic in several human studies, making it a promising noninvasive urinary biomarker of AKI [227].

Neutrophil gelatinase-associated lipocalin (NGAL) is rapidly expressed in kidney cells in response to several forms of stress and are detected in low levels in serum of healthy individuals. NGAL filtered in the glomerulus is reabsorbed in the proximal tubule, resulting in only low levels in urine. Decreased proximal tubule absorption of filtered NGAL in AKI, in combination with increased production by distal tubular epithelial cells, leads to increased NGAL levels in urine. Tumors, sepsis, and leukocyturia, without any renal injury, can also result in increased NGAL levels, making them important confounders when interpreting NGAL data. Though reference values have been established in healthy adults (higher levels in women and increasing with age), progress with this biomarker will require specific informative cutoff values for the different clinical situations [228, 229].

Additional urinary biomarkers identified as detecting early kidney injury include cystatin C, N-acetyl β-D-glucoseaminidase (NAG), and interleukin 18. Any individual biomarker, if accepted as a biomarker of renal injury, can help overcome the shortcomings of serum creatinine as a measure of AKI, which include the inability to differentiate early between established kidney injury and reversible hemodynamic changes resulting in physiologically reduced GFR. Since each biomarker has unique strengths and weaknesses, using a marker panel might be more reliable in determining the type and severity of kidney injury, and allow for more precise prognosis models [230, 231].

Repair Mechanisms

Just as multiple pathways to cell injury are activated in AKI, the repair processes would be expected to be multifaceted. The mechanisms to renal recovery, then, might include: a) endogenous repair of sublethally injured cells, b) proliferation of surviving renal tubular cells, or c) replacement of dead cells by stem cells. The rapid initiation of repair in injured tubules supports the theory of endogenous repair of sublethally injured cells [232–234]. A deep understanding of the recovery process could identify interventions that augment cellular repair to restore kidney function more quickly and fully.

Heat Shock Proteins

Heat shock proteins (Hsps), also called stress proteins, are elaborated in cells in response to a variety of injurious stimuli. Hsps function as protein chaperones and are essential to basic cell function and survival by providing routine assistance in intracellular protein handling [235]. The two stress protein families most studied in AKI are the Hsp70 and the small Hsp 25/27 class. Heat shock transcription factor-1 (HSF-1) activation, the initiator of the stress response, is rapid after onset of renal ischemia. Significant Hsp induction in the kidney soon follows [83, 236, 237].

Renal tubule cells that suffer loss of structural integrity in AKI regain their normal architecture and polarity through remodeling [104]. Recycling of damaged proteins by the chaperone capacity of Hsps appear to be central to the repair process. Hsp72 binds specifically to disrupted Na/K-ATPase, and Hsp25/27 binds specifically to disorganized actin filaments in renal epithelial cells injured by ischemia or ATP depletion [238–240]. Furthermore, both Hsp72 and Hsp25/27 have been shown to be protective against injury from in vitro ischemia in proximal tubule cells [239–241]. Hsp72 and 25/27 appear to work synergistically to preserve renal cell structure [242].

Supporting the in vitro studies, proximal tubule cells isolated from transgenic mice that overexpress human Hsp27 are tolerant to hydrogen peroxide injury. However, these same mice manifest more severe ischemic AKI in vivo [243]. The worse renal outcome in vivo was attributed to increased pro-inflammatory gene expression with increased neutrophil and lymphocyte infiltration into the kidney early after ischemia. In contrast to systemic Hsp27 overexpression, selective renal overexpression of Hsp27 protected against ischemic renal injury in vivo [244]. With renal-specific Hsp27 overexpression, protection against AKI was attributed to decreased neutrophil infiltration of kidneys and reduced pro-inflammatory gene expression.

A more actively primed stress response may contribute to the phenomenon, long recognized by clinicians, that the immature kidney appears to be resistant to insults that produce profound AKI in older patients. As in patients, immature rat kidneys are more resistant to ischemic injury than their mature counterparts. The neonatal rat kidney has increased constitutive activity of HSF-1 compared with mature animals, with accompanying increased expression of Hsp72 [245, 246]. Blocking HSF-1 function reduces Hsp72 expression and the tolerance of isolated immature renal tubules to anoxia [245–247]. Stress proteins, then, appear to be central to the resistance of immature kidneys to ischemic or anoxic injury.

It was thought, therefore, that the cellular stress response regulated by HSF-1 would also be central to resistance to in vivo AKI in mature animals. It was a surprise, then, when it was found that HSF-1 knockout mice, with absent Hsp induction following renal ischemia, are resistant to developing AKI after an ischemic insult [248]. HSF-1 knockout mice were found to have an increased baseline level of immunomodulatory Foxp3+ regulatory T cells resident in the kidney. This appears to prevent the development of usual features of AKI seen in kidneys shortly following an ischemic insult, specifically the pro-inflammatory environment and the typical medullary vascular congestion.

Thus, Hsps clearly provide protection at the cellular level in renal tubules, but the systemic effect of inducible Hsps may instead be harmful in the setting of an in vivo ischemic insult. Detrimental effects of inducible Hsps, outside of the renal tubule and in the microvasculature, may conflict with their beneficial effects within renal tubule cells subjected to ischemia by facilitating pro-inflammatory mechanisms of renal injury. The most recent studies, then, uniquely connect the rapidly inducible cellular stress response, regulated by HSF-1, to pathways of inflammation in ischemia-induced AKI.

Regeneration: Growth Factors

It has long been suspected that growth factors, produced locally or exogenous to the kidney, contribute to proliferation and redifferentiation of the tubule epithelial cell following AKI (Fig. 1). Many studies have supported this concept. These include epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), parathyroid hormone–related peptide (PTHrP), basic fibroblast growth factor, osteopontin, neural cell adhesion molecule, transforming growth factor beta 1 (TGF-β), Klotho, and fibroblast growth factor 23 [233, 249–252]. The plethora of growth factors that appear to have a role in renal recovery in AKI raises questions about each factor's specific site of action, potential interaction or overlap of activity between separate growth factors, the specific cellular processes each factor impacts, and the relative importance of each factor in the recovery from AKI. It is not a surprise that growth factors are found to be active in several pathways pertinent to AKI.

Growth factor function may be intimately linked to processes of apoptosis. In the rat model of AKI, expression of pro- and antiapoptotic members of the Bcl-2 gene family (Bcl-2, Bcl-X (L), Bax) are related to tubule expression of EGF, IGF-1, and TGF-β, all growth factors thought to be reparative in AKI [251]. The study suggested that the distal tubule is resistant to ischemic injury through antiapoptotic effects of Bcl-2 genes, and its survival allows expression of growth factors critical not only to the survival and regeneration of its own cell population (autocrine action), but also to the adjacent ischemia-sensitive proximal tubule cells (paracrine action).

AKI in rats increases expression of transcription factors Ets-1 and Wnt-4 that are in pathways involved in cell cycle progression [253]. Nephrogenic proteins basic fibroblast growth factor (bFGF), vimentin, neural cell adhesion molecules, paired homeobox-2, bone morphogene protein-7, Noggin, Lim-1, Engrailed, Smad, phospho-Smad, hypoxia-inducible factor-1 alpha (HIF-1 alpha), vascular endothelial growth factor (VEGF), and Tie-2 are all induced by renal ischemia in a pattern similar to that seen in normal renal development, and administration of bFGF-accelerated induction of many of those nephrogenic proteins during recovery [254, 255]. HIF-1 alpha is rapidly activated by renal ischemia, and growth factors VEGF and erythropoietin, both regulated by this transcription factor, appear to have important effects on renal epithelial cells and endothelial cells in the renal microvasculature. Erythropoietin administration prior to renal ischemia protects the kidney against the injury through mechanisms that limit apoptosis [256].

The relative importance of the many growth factors identified to be active in AKI has rarely been addressed. One study, however, directly compared the effect of two of these growth factors in AKI using a transgenic mouse model with targeted overexpression in the proximal tubule of HGF in one strain, and PTHrP in another strain. The strain with increased HGF in the proximal tubule had a fourfold increase in tubule cell proliferation and a threefold decrease in apoptotic tubule cell death, along with rapid recovery from ischemic injury, compared with either the mice with targeted proximal tubule overexpression of PTHrP or background strain control mice [257]. This study confirmed the importance of HGF during the recovery process, but raises questions about the role of rapid PTHrP induction in the proximal tubule after renal ischemia. By extension, it also raises questions about all of the growth factors identified as being induced by renal ischemia whose functional effects are yet to be defined. Some may be beneficial. Some, such as TGF-β, may have detrimental effects in the long term. A delicate balance of these factors may determine whether tissue remodeling following renal injury results in restitution of normal architecture or proceeds to a dead end of irreversible fibrosis.

Regeneration: Stem Cells

With the advent of stem cell research, the possibility that some form of stem cell might be integral to kidney repair after AKI rapidly came to the fore. Embryonic and nonembryonic/adult stem cells, the latter from several sources, can differentiate into more than one type of specialized cell. Mesenchymal stem cells residing in the bone marrow can differentiate into a variety of mesenchymal tissues in vitro and in vivo. Tissue-specific progenitor cells may reside in any organ and can differentiate into a range of cells as the need arises. Each of these potential sources of stem cells have been studied to determine the contribution to renal repair following AKI.

Initial studies supported the concept of bone marrow–derived stem cells (BMDSC) homing to ischemia-injured kidneys, and differentiation of noninflammatory bone marrow derived cells into renal tubule cells [258–260]. However, subsequent studies showed that BMDSC contribute minimally to repopulation of tubular epithelia [261–265]. It now appears clear that repopulation of denuded tubules after AKI comes from proliferation of surviving intrinsic kidney cells, rather than from exogenous or endogenous stem cells [263].

Nevertheless, BMDSC have a role in the repair process. The severity of renal injury in bone marrow–ablated mice was worse than without ablation and was reversible with infusion of lineage-negative bone marrow cells [259]. Although mesenchymal stem cells (MSCs) may not engraft into renal tubules, they have functional effects in AKI, such as blunting the rise in serum creatinine and altering pro-inflammatory cytokine and growth factor expression [261, 266]. These findings suggest that protection by MSC may be mediated by paracrine or endocrine actions [266, 267]. Furthermore, mobilizing endogenous BMDSCs may be a therapeutic option in AKI using stem cell factor or colony-stimulating factors (macrophage-CSF or granulocyte-CSF) [268, 269]. The beneficial effects of these agents might be through ameliorating injury pathways detailed earlier in this chapter, along with affecting recovery pathways. The basic science studies of stem cells in AKI are being translated into clinical studies. Thus far, phase 1 clinical trials using allogeneic MSC show that they are safe, but in a phase II trial there was no evidence of protection against AKI in patients undergoing on-pump cardiac surgery [270, 271].

Long-Term Sequelae

The focus in the clinical realm, and in experimental models of AKI, typically is on the severity of the acute renal manifestations and the speed and completeness of early recovery. Little attention is commonly given to long-term sequelae in the kidney following an episode of AKI, since most patients who survive the triggering event appear to recover renal function completely. Decades ago it was found that many adult patients who suffered from acute renal failure never recovered renal function completely, manifested by decrements in GFR and urinary concentrating ability months to years later [272–276]. Prior to 2003, these studies were few in number, examined relatively small sample sizes and with varying definitions for both AKI and progression. Potential causative links between AKI and chronic kidney disease (CKD) were not investigated and no serious consideration was given to understand long-term expectations or develop plans for management of patients following AKI.

However, in the last 15–20 years there has been a dramatic increase in research on the chronic effects of AKI [19, 277–280]. Recent studies using large databases have found significant correlation between AKI and progression to CKD and end-stage renal disease (ESRD) [281]. Meta-analysis found an 8.8-fold increase risk for CKD and a 3.3-fold increase risk for ESRD in surviving AKI patients after hospital discharge [282]. In addition, the finding of significant and progressive renal insufficiency in adolescence and early adulthood after recovery from AKI as a neonate is of particular interest. Though the neonatal kidney may be more resistant to AKI, it may be more prone to develop late sequelae once AKI has been established [19, 277–280].

The cause of progression to CKD following AKI is not clearly identified. Animal models indicate that there is a lack of fidelity in the repair responses following injury. Renal repair can be classified as either adaptive or maladaptive, and both may occur simultaneously. Maladaptive repair is the development of fibrosis in the tubular and interstitial compartments in response to acute kidney injury.

The underlying pathophysiology that predisposes to the development of CKD following AKI is not clearly defined, but has been subject to intensive investigation in recent years. Animal models of AKI with extended recovery from what was historically studied (months vs. days) suggest a lack of fidelity in the repair responses which fail to restore the kidney to its pristine, pre-injury state. These alterations may be the link between acute injury and subsequent development of CKD and hypertension [19]. An evolving view is that ineffective or maladaptive repair occurs in the tubular and interstitial compartments following AKI allowing for the development of interstitial fibrosis [283].

Several pathophysiological processes may contribute to maladaptive repair (see Fig. 6). Incomplete tubular repair following AKI leading to CKD has been a primary topic in the AKI-to-CKD transition. In severe cases, or following repeated insults, tubular repair may be absent leading to disconnection of intact glomeruli from residual tubule segments, with subsequent decline in overall renal function [284, 285]. However, incomplete repair may also manifest as persistent dedifferentiation of a subpopulation of tubule cells or as tubular cells that have initiated proliferation but become growth arrested in the G2 phase of the cell cycle. These cells are thought to produce profibrotic factors that stimulate the adjacent interstitium to develop fibrosis [286–288]. Epigenetic alterations during repair may facilitate these changes through histone modifications, DNA methylation, or chromosomal conformational changes that regulate gene expression. Histone deacetylase inhibitors accelerate recovery and decrease post-injury fibrosis after ischemia and reperfusion [289]. Single-nuclei RNAseq and advanced informatics have newly identified a population of cells that transform into what has been termed “failed-recovery proximal tubule cells” (FR-PTC). This small population of cells persists following AKI and have been found to have activated profibrotic and inflammatory pathways. A similar population of FR-PTC was identified in human kidney allografts [290].

In addition to altered tubules, reduction in capillary density is found following acute kidney injury in multiple animal models and in humans [291]. The reduction in renal capillary density is

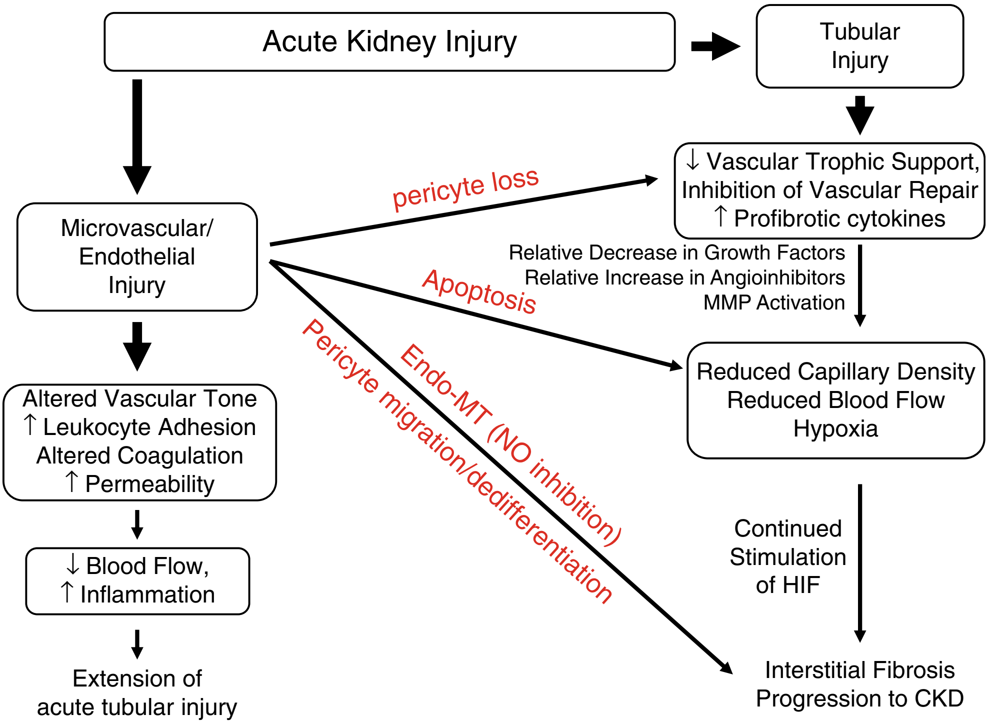
Acute Kidney Injury
Tubular Injury
↓ Vascular Trophic Support,
Inhibition of Vascular Repair
↑ Profibrotic cytokines
pericyte loss
Microvascular/ Endothelial Injury
Relative Decrease in Growth Factors
Relative Increase in Angioinhibitors
MMP Activation
Apoptosis
Pericyte migration/dedifferentiation
Endo-MT (NO inhibition)
Reduced Capillary Density
Reduced Blood Flow
Hypoxia
Altered Vascular Tone
↑ Leukocyte Adhesion
Altered Coagulation
↑ Permeability
↓ Blood Flow,
↑ Inflammation
Continued Stimulation of HIF
Extension of acute tubular injury
Interstitial Fibrosis
Progression to CKD

Fig. 6 *Potential pathways from AKI through microvascular injury to chronic kidney disease (CKD).* Renal microvascular injury in AKI, in addition to extending the acute tubule injury, contributes to development of CKD through several possible mechanisms. Microvascular injury triggers inflammation with increase in pro-fibrotic cytokines and inhibition of vascular repair. Subsequent microvascular dropout can contribute to ongoing local hypoxia. Chronic hypoxia along with endothelial-mesenchymal transition (EndoMT) may then promote interstitial fibrosis. (From Ref. [19])

thought to contribute to local hypoxia, which is considered a major stimulus driving interstitial fibrosis [291]. Thus, strategies to mitigate renal vascular loss or local hypoxia might prevent progressive CKD following an episode of AKI. Renal vascular loss is associated with a reduction of renal VEGF expression, and exogenous VEGF-121 supplementation preserves capillary density and mitigates progression to CKD in rats [43, 292]. The capillaries of the kidney appear to have limited repair potential, and the ability to reverse established rarefaction is not well described [293]. Nevertheless, the use of novel VEGF-derived chimeric proteins, myeloid-derived-angiogenic cells, or endothelial colony-forming cells might lead to enhancing renal vascular recovery [67, 294].

In the absence of vascular repair, renal vasodilators might reduce hypoxia and prevent CKD progression. For example, administration of L-arginine postischemia improved chronic renal blood flow, limited hypoxia, and prevented CKD progression without restoring capillary density [43]. Another potential strategy to prevent CKD after AKI is through activating intrinsic defensive mechanisms against hypoxia and oxidative stress. HIF activation prior to induction of AKI lessens the acute injury and prevents the later development of fibrosis [295, 296]. Pharmacological Nrf2 activation induces antioxidative genes and ameliorates AKI in mice [297, 298]. Although promising, it remains to be determined whether Nrf2 activation can prevent the transition of AKI to CKD.

Fibroblasts, myofibroblasts, and the persistent influence of inflammatory macrophages and lymphocytes contribute to late alterations in the interstitial compartment after AKI [288]. The expansion of the interstitial space appears to follow several processes: pericyte activation and conversion into a myofibroblast-like state [299], invasion of fibrocytes [300], and endothelial-mesenchymal cell transition [291]. Fibroblasts and myofibroblasts are considered the primary cells responsible for interstitial scar formation. Persistent oxidative stress or the production of profibrotic factors (e.g., TGF-β, CTGF, and PDGF-B) derived from either damaged PTCs, FR- PTC, or infiltrating macrophages or lymphocytes may promote progressive interstitial fibrosis [288, 301]. Strategies to block chronic macrophage responses can block progressive kidney disease following AKI [302]. In addition, persistent lymphocyte expression, particularly T helper 17 cells, has been shown during CKD progression following AKI. Inhibition of lymphocytes with mycophenolate attenuates the transition to CKD following ischemia-induced AKI in rats [303].

Conclusion

The study of acute kidney injury that leads to clinical acute renal failure continues to advance. The progressive discovery of the many facets in the pathogenesis of the injury, markers of the injury, and potential recovery mechanisms provides hope that therapy targeted to the kidney injury specifically, along with ongoing advances in supportive therapy, will substantially improve survival and wellness of patients affected by acute kidney injury. Suggested by the many interactive pathways in AKI outlined here, both for ischemia- and sepsis-induced AKI, no single intervention alone is likely to alter outcome substantially. An integrated approach may be required to limit injury or facilitate renal recovery. Having a clear understanding of the pathogenesis of acute kidney injury from a particular renal insult should help direct when to intervene with a specific therapy, and which intervention would be most likely to benefit the patient.

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Evaluation and Management of Acute Kidney Injury in Neonates

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Jennifer G. Jetton and Enrico Vidal

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Abstract

Critically ill neonates are at risk for acute kidney injury (AKI) and associated complications including fluid overload, longer mechanical ventilation time, increased hospital length of stay, and death. While the general approach to the evaluation and management of AKI in babies is similar to that in older children and adults, optimal care of these fragile patients requires an understanding of the aspects of neonatal physiology and disease unique to this patient group. Neonatal-specific high risk-conditions include extreme prematurity, perinatal asphyxia, necrotizing enterocolitis, and hemodynamically significant patent ductus arteriosus. In addition, neonates exhibit a renal physiology that changes as they transition from intra- to extra-uterine life, one that varies also between term and preterm infants, and creates challenges for interpretation of kidney function biomarkers such as serum creatinine and urine output. Patient heterogeneity in terms of gestational age and size poses additional challenges for the standardization of AKI definitions and monitoring as well as for provision of kidney support therapy when needed. Medical management has historically been the cornerstone of therapy due to technical barriers associated with providing kidney support therapy to these small patients, but advances in technology are reshaping the indications, timing, and risks and benefits of dialysis provision in this setting. New research is highlighting the interplay between AKI and other organs, especially the lungs and brain. The risk for chronic kidney disease, especially in extremely premature infants with and without a history of AKI, is becoming increasingly clear and highlights the need for long-term follow-up.

Keywords

Acute kidney injury · Neonate · Oliguria · Fluid balance · Biomarkers · Kidney support therapy

Synonyms

Acute kidney injury, acute renal failure; Kidney support therapy, renal replacement therapy; Newborn, neonates

Introduction

Critically ill newborns are at risk for acute kidney injury (AKI) and related complications including morbidity, mortality, and increased hospital length of stay, similar to older patients. While some of the causes of AKI are the same in neonates as they are in older children and adults (e.g., hypotension, sepsis, medication nephrotoxicity), other high-risk conditions and differing pathophysiology are unique to this patient group. In addition, the diagnosis of AKI in neonates using standard AKI biomarkers and definitions is made more challenging by the unique neonatal renal physiology during the first week of life. Moreover, the wide range in gestational age (GA) and size – from extreme prematurity at 22 weeks gestation to term infants at 40+ weeks – creates significant heterogeneity within the neonatal intensive care unit (NICU)population. The extremes in gestational age and size create challenges for the use of kidney Replacement therapies (KRT), most of which have been designed and sized for adults but only adapted for children, when necessary. In this chapter, we provide an overview of the evaluation and management of AKI in neonates including discussion of mechanisms and high-risk conditions, strengths, and limitations of available kidney function biomarkers, medical management strategies, and KRT options.

Acute Kidney Injury in the NICU: Scope of the Problem

Both single-center and a large multicenter cohort studies have demonstrated that AKI is common in critically ill neonates, ranging from 30% to 40% depending on the sample and associated comorbidities [1–3]. In addition, neonates with AKI have higher mortality rates than those without AKI. A large multinational, multicenter study of neonates, the Assessment of Worldwide Acute Kidney Injury Epidemiology in Neonates (AWAKEN) showed an overall AKI incidence of 30%, with a U-shaped distribution according to GA (37% in term infants, 18% in 29–36 weeks, and 48% in <29 weeks GA) [1]. Other samples from developing countries show similarly high rates of AKI incidence and associated mortality. Mwamanenge [3] identified an AKI rate of 31.5% in their cohort from Tanzania where AKI was primarily related to sepsis, pneumonia and use of gentamicin. In the AWAKEN study, two-thirds of the AKI events happened early in the course (first postnatal week), with the other 1/3 happening after 7 days of life [4, 5]. Importantly, 28% of those babies who had an early AKI event had at least one additional AKI episode later in the hospital course.

AKI Definitions and Biomarkers

The AKI definition for neonates remains an area of ongoing research and debate. Standardized AKI definitions have been derived from adult data and extrapolated for use in children and infants. Some of the variability in the rates of AKI reported in neonatal studies is attributable to the variety of AKI definitions used, ranging from arbitrary cut-offs (e.g., serum creatinine ≥1.5 mg/dL) to categorical frameworks defining incremental increases in serum creatinine and decreases in urine output from established baselines. The most widely accepted definition currently is a modification of the Kidney Disease: Improving Global Outcomes (KDIGO) definition of AKI [6] (Table 1).

The challenge of establishing an AKI definition for neonatal cohorts is related to the unique renal physiology of neonates, especially in the first week of life when neonates are in a relative polyuric state. As mentioned above, kidney function in neonates undergoes dynamic change following birth. Cardiac output and resultant renal blood flow increase rapidly over the first days and weeks of life. Glomerular filtration rate (GFR) in the term infant increases from 10–40 ml/min/1.73 m^2 in the first 1–2 weeks of life to the expected adult GFR 100–120 ml/min/1.73 m^2 by

Table 1 Modified KDIGO AKI definition for neonates [6]

Stage	Serum creatinine (SCr)	Urine output (UOP)[a]
0	No change in SCr or rise <0.3 mg/dL	>1 mL/kg/h
1	SCr rise >0.3 mg/dL within 48 h *or* SCr rise ≥1.5–1.9 × rise from baseline (previous lowest value) within 7 days	> 0.5 and ≤ 1 mL/kg/h
2	SCr rise ≥2–2.9 × rise from baseline	> 0.3 and ≤ 0.5 mL/kg/h
3	SCr rise ≥3 × rise from baseline *or* SCr ≥ 2.5 mg/dL *or* KRT initiation	≤ 0.3 mL/kg/h

KDIGO kidney disease: improving global outcomes, *AKI* acute kidney injury, *Mg/dL* milligrams per deciliter, *ml/kg/h* milliliters per kilogram per hour, *KRT* kidney support therapy

[a] For 24 h period

age 2 years [7]. This change occurs more slowly in preterm infants. Serum creatinine values, initially reflective of maternal levels, are therefore expected to decline over the first week(s) of life rather than remain in a steady state, making the application of standard AKI definitions problematic. In addition, early transient increases in serum creatinine may also be expected, especially in preterm infants, due to avid reabsorption of creatinine [8]. Some authors have proposed using absolute serum creatinine increases (e.g., >0.3 mg/dL) that vary by gestational age [9], while others have advocated using the rate of serum creatinine decline over the first week as an indication of impaired kidney function [10].

Oliguria, while recognized as an important and often under-recognized marker of AKI [11], is similarly difficult to utilize as a marker of AKI in the newborn. Babies undergo an obligate diuresis following birth. Urinary concentrating ability, especially in preterm infants, is immature such that neonates may sustain an AKI event while maintaining urine output above traditional AKI thresholds (e.g., <0.5 ml/kg/h). A higher urine output threshold <1.5 ml/kg/h has been evaluated as a reasonable predictor of mortality in one NICU cohort [12].

In addition to these limitations of serum creatinine and urine output for diagnosing AKI in neonates, they are also imprecise in their ability to distinguish functional changes from evidence of true injury/damage and to identify AKI early enough to intervene [13]. Thus, other kidney biomarkers are being evaluated in neonatal cohorts. Cystatin C (CysC), for example, is a cysteine protease inhibitor produced by all nucleated cells, is freely filtered by the glomerulus, and completely reabsorbed and catabolized by the proximal tubule [14, 15]. Because levels are not affected by hydration status, diet, or gender, both urine and serum CysC have been evaluated for their ability to identify AKI more reliably than serum creatinine, including in studies of term and preterm neonates [16, 17]. Other novel urinary biomarkers are under investigation in neonates, but not yet ready for broad clinical use. Importantly for neonates, the establishment of reference ranges is critical as levels vary by gestational age, postnatal age, sex, and birthweight [13, 18, 19].

Urine output response to a single dose of furosemide has been evaluated as a functional biomarker in critically ill adults, showing ability to predict progression to AKI stage 3 (“furosemide stress test”) [20]. Furosemide reaches the loop of Henle via tubular secretion and thus should have decreased efficacy in patients with AKI. Urinary response to furosemide has been evaluated in neonates undergoing cardiac surgery [21, 22]. In one study, a urine output response of >1 ml/kg/h following a single dose of furosemide (0.8–1.2 mg/kg between 8 and 24 h after surgery) identified those babies who were unlikely to go on to develop postoperative AKI (negative predictive value, 97%) [21].

Because of limitations of kidney function biomarkers in neonates, awareness of high-risk conditions is critical for detecting AKI early and preventing AKI-related complications. As mentioned above, many mechanisms of injury are the same in neonates as in older patients, but there are important conditions specific to this patient group. Some of the more well studied in terms of their association with AKI are described below.

High Risk Conditions

Prematurity and Low Birth Weight (LBW)

Babies born prematurely deliver prior to the completion of nephrogenesis, a process that typically occurs from 5–8 weeks to 34–36 weeks gestation, with the majority of nephrons forming in the third trimester [23]. Thus, these infants are born with less nephron endowment and with nephrons that are abnormal in structure and function. Autopsy studies of premature kidneys show that nephrogenesis continues briefly in the newborn period, but the nephron layers that are laid down are abnormal and at risk for additional injury [24, 25]. Decreased nephron mass plays an important role not only in AKI, but in long-term kidney health as well.

Single center data confirm that very low birth weight (VLBW) babies are at risk for AKI, and those with AKI have higher risk for mortality [2, 26, 27]. An ancillary study of the large multicenter Pre-term Erythropoietin Neuroprotection Trial (PENUT) evaluated 942 extremely premature infants <28 weeks GA to describe the epidemiology of AKI, relevant AKI outcomes and progression to CKD in population. This Recombinant Erythropoietin for Protection of Infant Renal Disease study (REPAIReD) also demonstrated AKI rates *increased* with *decreasing* gestational age and lower birthweight [2]. Indeed, lower gestational age and birthweight are frequently identified risk factors for higher rates of AKI across studies [28–30]. Other risk factors in this group include hemodynamically significant patent ductus arteriosus (hs-PDA) and necrotizing enterocolitis (NEC), higher severity of illness scores, and early symptomatic hypotension requiring volume resuscitation and/or pressor support [4, 28].

Term Infants

Term infants are also at risk for AKI. Thirty-seven percent of neonates with AKI in AWAKEN were in the ≥36 weeks GA group, a lower rate than in the extremely premature group (52% in <29 weeks GA), but higher than in the middle group (18.% in 29–26 weeks GA) [1]. Similar rates (35% and 46% depending on the AKI definition used) were documented in a sample of 80 infants (median GA 38 weeks) admitted to a pediatric intensive care unit either from home or the NICU [31]. Factors associated with AKI in this sample included younger age (median 3 vs. 12 days), higher serum sodium concentrations prior to AKI, diagnosis of persistent pulmonary hypertension (PPHN), history of umbilical catheters, bacteremia, transfer from the NICU, and support with extracorporeal membrane oxygenation (ECMO).

Perinatal Asphyxia and Hypoxic-Ischemic Encephalopathy (HIE)

Neonatal hypoxic-ischemic encephalopathy (HIE) occurs secondary to a period of inadequate cerebral blood flow and oxygen delivery, either in utero or in the perinatal period, as the result of compromised placental or pulmonary gas exchange [32]. In addition to cerebral injury, babies with perinatal asphyxia are at risk for multiorgan dysfunction. Improvements in both survival and neurodevelopmental outcomes have come about with the use of therapeutic hypothermia (whole body cooling) for infants 36 weeks gestational age and above [33].

Neonatal AKI is well documented in this setting [34–37]. Possible mechanisms include ischemia-reperfusion injury, other underlying comorbidities such as sepsis, and use of nephrotoxic medications [32]. Several studies document an association between AKI and higher rates of morbidity, mortality, and hospital length of stay in this setting. Moreover, worse neurodevelopmental outcomes have also been associated with the occurrence of AKI [35, 36]. In terms of AKI mitigation, several studies have examined the protective effects of methylxanthines (theophylline and aminophylline) in babies with perinatal asphyxia and are discussed further below. Therapeutic hypothermia has not been examined specifically as a kidney-protective strategy and is now standard of care in high-resource settings.

Necrotizing Enterocolitis (NEC)

Necrotizing enterocolitis (NEC) is a life-threatening, inflammatory intestinal condition that occurs in premature infants [38]. NEC is associated with AKI for multiple reasons including the underlying severe systemic inflammatory response, sepsis, and hypotension [28, 39–41]. AKI rates are high in these patients, reaching >50% in some studies [28, 40]. Babies with NEC and AKI have higher mortality rates than babies with NEC who do not develop AKI. These critically ill infants require broad spectrum, empiric antimicrobial coverage to cover for pathogens associated with late-onset sepsis and for anaerobic infections that occur as the result of intestinal perforation, adding to the AKI risk. These babies require careful kidney function, antibiotic level, and fluid balance monitoring.

Patent Ductus Arteriosus (PDA)

The ductus arteriosus plays a central role in fetal circulation in utero, connecting the main pulmonary artery to the descending aorta and allowing blood to bypass the fetal lungs. High circulating levels of prostaglandin E2 (PGE_2) promote smooth muscle relaxation and maintain the patency of the ductus. In full-term infants, the ductus arteriosus closes within a few hours after birth, but in premature infants this process may be delayed [42]. Depending on the size of the ductus, the PDA may become hemodynamically significant (hs-PDA), shunting blood away from systemic circulation, compromising perfusion to the gut and kidneys, and leading to pulmonary hypertension and right heart failure [43]. In addition to hypoperfusion-related AKI, a hs-PDA may be associated with adverse outcomes including bronchopulmonary dysplasia, pulmonary hemorrhage, NEC, intraventricular hemorrhage (IVH), and poor neurodevelopmental outcomes [42]. Treatment of the hs-PDA includes both medical and surgical options. Medical management is aimed at lowering PGE_2 levels such as with the use of nonsteroidal anti-inflammatory drugs (NSAIDS) like indomethacin and ibuprofen. AKI may arise as the result of hemodynamic changes and impact on renal perfusion, the vasoconstriction, and impact on renal blood flow of the NSAIDs. Because of the known potential nephrotoxicity of NSAIDs, alternatives such as acetaminophen/paracetamol have been evaluated, with some evidence showing similar efficacy in ductal closure rates and fewer side effects using this therapy compared with traditional indomethacin [44]. Management decisions regarding timing and appropriate treatment for hs-PDAs present clinical challenges for the medical team [45].

Congenital Heart Disease and Cardiac Surgery-Associated AKI (cs-AKI)

The occurrence of AKI in babies undergoing surgery for congenital heart disease (cs-AKI) is well documented [21, 29, 30, 46, 47]. Risk factors include lower age and weight at the time of surgery, higher surgical complexity scores, longer cardiopulmonary bypass, and cross-clamp times [21, 22, 29, 30, 46]. As in other patient groups, AKI in this setting is associated with worse outcomes including longer length of stay, longer time to extubation, more ventricular dysfunction, need for ECMO, and higher rates of mortality [30, 46]. The occurrence of cs-AKI also has been linked with the development of chronic kidney disease (CKD) [29]. Since the timing of the kidney insult(s) (e.g., inflammation associated with cardiac bypass, intraoperative hypotension, ischemia-reperfusion injury) related to cardiac surgery is known, it is reasonable to consider options for AKI therapeutic interventions in this context. Several therapies including corticosteroids, dexmedetomidine (a selective α2-agonist that may attenuate inflammation and subsequent ischemia-reperfusion injury), fenoldopam (a selective dopamine-1 receptor agonist that increases renal blood flow through vasodilation of renal and splanchnic vasculature), acetaminophen, and aminophylline have been evaluated as preventive measures, but none have demonstrated clear efficacy in their ability to prevent cs-AKI [48].

Congenital Diaphragmatic Hernia (CDH)

Congenital diaphragmatic hernia (CDH) is a defect that allows abdominal contents to enter the chest cavity leading to variable degrees of pulmonary hypoplasia and pulmonary hypertension. Many of these infants are critically ill at birth, requiring initial support with extracorporeal membrane oxygenation (ECMO). Not surprisingly, this condition has also been associated with high rates of AKI, with reported rates ranging from 37% in all infants with CDH to 71% in those requiring ECMO support [49–51]. AKI in this context is also associated with higher mortality rates.

Extracorporeal Membrane Oxygenation (ECMO)

Extracorporeal membrane oxygenation (ECMO) is a form of advanced mechanical life support for patients with severe but reversible forms of respiratory and cardiac failure refractory to conventional management. High rates of AKI have been documented in critically ill neonates requiring support with this therapy [50, 52, 53]. Patients on ECMO who develop AKI have a longer ECMO duration [52] and higher rates of mortality, with risk of mortality increasing with increasing AKI severity [50]. AKI appears to happen early in the ECMO course, often within the first 48 h of therapy initiation. Neonates supported with ECMO have many potential AKI risk factors including hemodynamic instability, multiorgan failure, hypoxia/asphyxia, sepsis, and shock that occur both prior to and during the ECMO run, and it has been suggested that the ECMO circuit itself may play a role in the inflammatory response [50, 52, 54].

Surgical Procedures

Admission with need for a surgical procedure was identified as a risk factor for both early and late AKI in the AWAKEN cohort, though AWAKEN study did not examine the temporal relationship between the procedure and the AKI event [4, 5]. A retrospective study of 160 neonates by Wu et al. [55] identified AKI following surgical procedures in 33.8% of the sample. AKI was associated with higher rates of mortality, with increasing mortality rates associated with greater severity of AKI events (stage 1–9.7%, stage 2–23.1%, and stage 3–40% mortality). Longer operative time (>120 min), lower gestational age and birthweight, sepsis, and diagnosis of necrotizing enterocolitis were all associated with AKI development. Median time to diagnosis of AKI was 38.5 h after the surgery or within 23 h for patients with sepsis.

Mechanisms

Medication-Induced AKI

Many of the medications used in the NICU to treat the conditions described above have nephrotoxic potential. Indeed, exposure to nephrotoxic medications is widespread, with over 80% of premature infants receiving at least one nephrotoxic medication during their NICU stay [56, 57]. There is not always a reasonable, non-nephrotoxic alternative to some of these medications, and whether the AKI that occurs in these settings is related to the underlying condition, the nephrotoxic medication, or a combination is not always clear. How to balance the risks and benefits of treating the condition with the AKI risks associated with the treatment itself continues to be a significant challenge for the teams caring for these infants [45]. Understanding the mechanism of injury from both the underlying condition and the medications is important for developing effective AKI monitoring and mitigation strategies. Table 2 includes a summary of medications commonly used in the NICU and their mechanisms of injury.

Vascular/Ischemic Injury

Renal Vascular Thrombosis

With a combination of lower renal perfusion pressure and blood flow, small renal vessel diameter, and susceptibility of the neonatal hemostatic system to imbalance, neonates carry a high risk for thromboembolic complications involving the renal vasculature [58]. Many conditions that occur during the perinatal period are risk factors for renal vascular thrombosis, including perinatal asphyxia, volume depletion, hypotension, placement of indwelling catheters, polycythemia, and activation of the renin-angiotensin-aldosterone system (leading to vasoconstriction). Additionally, maternal factors such as preeclampsia, diabetes, and autoimmunity have also been associated with these events [59]. Cases of renal arterial thrombosis (RAT) have been associated with arterial catheters (especially when placed for prolonged periods), prematurity, low birth weight, sepsis, and retinopathy of prematurity [60]. Renal vein thrombosis (RVT) is much more common than RAT, though neither occur with great frequency. RVT seems to present more frequently in males and is more commonly unilateral, and with the classic triad of gross hematuria, palpable flank mass, and

Table 2 Commonly used medications in the NICU associated with AKI

Drug name	Mechanism of action	Mechanism of injury
Acyclovir	Antiviral therapy that works by inhibiting virus DNA polymerase, which stops the spread of HSV in the body. Acyclovir has activity against HSV, VZV, and EBV	Tubular damage through crystallization and obstruction especially with low urinary flow
Amphotericin B deoxycholate (conventional)	Disrupts fungal cell wall synthesis by binding to ergosterol, leading to formation of pores that leak cellular components	Direct distal tubular toxicity and vasoconstriction. Signs of nephrotoxicity include hypokalemia, hyponatremia, metabolic acidosis, and hypomagnesemia as well as elevated serum creatinine
Gentamicin	Aminoglycoside that interferes with bacterial protein synthesis by binding to 30S ribosomal subunit resulting in a defective bacterial cell membrane	High binding affinity for the proximal tubular brush border membrane via the megalin complex. Signs of nephrotoxicity include electrolyte wasting but not usually a change in urine output. Alterations in kinetics seen in the setting of HIE [37]
Indomethacin	Nonselective COX inhibitor decreasing synthesis of prostaglandins which causes vasoconstriction and subsequent closing of PDA	Causes afferent arteriole vasoconstriction and reduced GFR
Piperacillin-Tazobactam	Inhibits bacterial cell wall synthesis, eventually leading to bacteria lysis	Piperacillin inhibits tubular secretion and clearance. Piperacillin and vancomycin combined may cause direct toxicity of proximal tubule cells
Vancomycin	Bactericidal glycopeptide that disrupts cell wall synthesis of Gram-positive bacteria	Unclear: piperacillin and vancomycin combined may cause direct toxicity of proximal tubule cells

DNA deoxyribonucleic acid, *HSV* herpes simplex virus, *VZV* varicella zoster virus, *EBV* Ebstein Barr virus, *PDA* patent ductus arteriosus, *GFR* glomerular filtration rate

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thrombocytopenia (though only a small fraction of cases with all three) [59]. RAT may be diagnosed during the work-up for neonatal hypertension. Diagnosis is confirmed with imaging such as ultrasound with Doppler. Findings suggestive of RVT include enlarged and echogenic kidneys with loss of corticomedullary differentiation and increased resistance in the renal arteries. The thrombus may extend into the inferior vena cava. Treatment may include heparin to prevent thrombus extension as well as thrombolytic agents in cases of more severe thrombus. While these RVTs may not be life-threatening, the majority result in some degree of permanent kidney injury (e.g., kidney atrophy) with resulting CKD in up to a third and end-stage kidney disease (ESKD) in some even with treatment [59].

Twin-to-Twin Transfusion Injury

Twin-to-twin transfusion syndrome (TTTS) is a rare disorder estimated to affect 10–20% of monochorionic twin gestations [61]. This serious condition occurs when there is unequal distribution of placental blood supply via shared vascular anastomoses and results in asymmetric fetal growth, postnatal morbidity including cerebral injury and respiratory distress syndrome, and mortality [61]. Twins are characterized as either the donor or recipient twin depending on the direction of the transfusion of blood from one to the other. AKI is well described as the donor twin experiences hypovolemia and hypotension-related ischemic injury. Advanced CKD/ESKD may also occur [62].

Renal Cortical Necrosis

Renal cortical necrosis is another rare but serious and irreversible condition that occurs as the result of profound ischemic injury. Catastrophic perinatal complications like placental abruption, congenital heart disease, and severe perinatal asphyxia occur during a critical period of change in renal blood flow with the transition to extrauterine life and put neonates at high risk for this event [63, 64]. Obstetrical emergencies that put both mother and baby at risk for AKI and renal cortical necrosis continue to be a clinical concern, especially in developing countries [63, 65], though the overall incidence of such events is declining.

Evaluation

History

Evaluation for AKI starts with a careful history searching for any of the events described above; more than one of these conditions may often be present. The prenatal course should be reviewed for reports of abnormal prenatal ultrasound findings (e.g., congenital anomalies of kidney and urinary tract (CAKUT), abnormal amniotic fluid levels), maternal medicine use (e.g., ACE-inhibitors, illicit drug use), perinatal complications suggesting fetal compromise including nonreassuring fetal status, placental abruption, maternal hemorrhage, pre-eclampsia/eclampsia, and intrauterine growth restriction. The delivery room course should be reviewed for the type of resuscitation needed as well as the time required to achieve stabilization. Delivery outside, and transfer to a tertiary healthcare setting (outborn delivery), has been associated with AKI in the first week of life [4]. Maternal factors such as receipt of steroids, hypertension/preeclampsia, and receipt of magnesium have been associated with lower incidence of AKI in the baby [4, 5, 28].

Physical Examination

The physical examination is critical for assessment of both volume and hemodynamic status. A primary goal of management is to ensure optimal renal perfusion as well as to prevent the consequences of fluid overload. Often the conditions described above (e.g., hs-PDA, perinatal asphyxia, NEC) are associated with hemodynamic imbalance that requires understanding of neonatal cardiovascular function and therapeutic intervention targeted at the disease pathophysiology in collaboration with the neonatology team [43].

Fluid Balance

Neonates are expected to undergo an obligatory diuresis during the first 10 days of life. Failure to do so, such as with the use of inappropriately high fluid intakes or in the setting of AKI, has been associated with increased risk of PDA [66], NEC [67], bronchopulmonary dysplasia [68], and prolonged mechanical ventilation. Achieving a negative fluid balance by postnatal day 7 has been associated with decreased risk for mechanical ventilation by that day in both preterm and term cohorts [69, 70]. As it has in older children and adults, fluid overload (FO) $>$ 10% in certain subgroups of neonates has been associated with worse outcomes, both in conjunction with and independent of diagnosed AKI [54, 71].

Early identification and management of FO is thus an important part of the evaluation and management of high-risk neonates, though critically ill infants are also at risk for complications related to volume depletion. While estimation of fluid balance is a focus of care, accurately determining the volume status of these babies can be difficult based on physical exam alone. Both weight-based [e.g., % weight change = (daily weight − birthweight)/birthweight × 100] and intake/output-based [e.g., % weight change = (intake − output from time of ICU admit)/admit weight × 100] formulas have been used for estimating cumulative fluid overload in neonatal and pediatric cohorts [72]. In addition, all fluid intake and loss should be reviewed. Even small volumes received via flushes and intermittently dosed medications may impact overall fluid balance, especially for premature and LBW infants. Common sources of losses for critically ill babies are described in Table 3. Some of these losses may be difficult to measure accurately. In addition, babies often do not have urinary catheters in place as a policy reduces infection risk. Urine output may be captured under “urine/stool mix” and may thus be hard to quantify. Good communication with bedside staff is critical. While urine output is often a

Table 3 Common AKI etiologies and risk factors in newborns

High risk conditions
Prematurity and low birth weight
Hemodynamically-significant PDA
Necrotizing enterocolitis
Perinatal asphyxia
Congenital diaphragmatic hernia
Support with ECMO
Need for surgical procedures (cardiac, thoracic, abdominal)
Outborn delivery
Treatment with ≥ 3 potentially nephrotoxic medications
Fluid loss and volume depletion
Gastrointestinal losses (emesis, diarrhea, NG, and enteral tube output)
Poor oral intake
Fluid losses from chest tubes, ventricular, and other drains
Excessive diuretic use
Congenital kidney disease with associated concentrating defects (e.g., dysplasia)
Polyuria following relief of urinary tract obstruction (“postobstructive diuresis”)
Inadequate renal perfusion
Congestive heart failure
Coarctation of the aorta
Treatment with ACE-inhibitors or NSAIDs
Hypoalbuminemia and low oncotic pressure
Increased abdominal pressure (e.g., with necrotizing enterocolitis)
Ischemic/vascular
Hemorrhage of any cause
Renal vessel thrombosis
Placental abruption/maternal hemorrhage
Twin-twin transfusion
Obstructive
Congenital malformations (posterior urethral valves, urethral stricture, neurogenic bladder)
Occluded urinary catheter
Medication-related urinary retention
Sepsis and other infections
Bacteremia, pyelonephritis, meningitis, congenital infections

PDA patent ductus arteriosus, *ECMO* extracorporeal membrane oxygenation, *NG* nasogastric, *ACE* angiotensin converting enzyme, *NSAIDs* nonsteroidal anti-inflammatory drugs

good marker of volume status, the kidneys of premature infants lack mature urinary concentrating ability and may have seemingly good urine output even in the context of volume depletion.

Other Innovations in Neonatal Intensive Care

Many of the conditions described above result in AKI because of hemodynamic instability and decreased renal blood flow. Given the limitations of kidney function biomarkers, use of other techniques to assess systemic and kidney perfusion could aid in the detection of at-risk neonates and help elucidate the underlying pathophysiology. Near-infrared spectroscopy (NIRS) provides a noninvasive, continuous method to monitor regional oxygenation in cerebral or somatic tissues, measuring oxygen saturation of hemoglobin in the sampling volume comprising venous, capillary, and arterial blood. NIRS is finding increasing use in NICUs for monitoring the kidney and in research studies as a tool for the early detection of AKI across a variety of settings in both term and preterm neonates [73, 74]. Changes in tissue oxygenation can identify renal hypoperfusion and hypoxia that may herald the development of AKI prior to changes in serum creatinine or urine output. Targeted neonatal echocardiography

(TnECHO) is another recent innovation being implemented in some centers [43]. The goal of TnECHO is to monitor changes in cardiac function and systemic perfusion in different disease states, guide appropriate vasoactive therapy, and assess response to therapeutic interventions. Optimization of hemodynamic status should benefit kidney function, though TnECHO has not been studied directly as an AKI prevention or mitigation tool.

Management

AKI Identification, Mitigation, and Prevention

Despite major advancements in our understanding of neonatal AKI, we still lack effective treatment strategies for this syndrome. Thus, current AKI care involves surveillance, early recognition, and prevention where feasible. Several awareness and intervention bundles have been developed in pediatric and adult cohorts [75–77], showing efficacy in improving identification and documentation rates of AKI events but not yet in reducing mortality [75]. An AKI bundle proposed for neonatal cohorts [78] is listed in Table 4. Increasing the frequency with which kidney function is monitored and documented in NICU is an important step in identifying AKI in these babies and ensuring appropriate follow-up [79, 80].

Table 4 Proposed neonatal care bundle to guide the evaluation and management of modifiable risk factors and complications of AKI

Monitor volume intake/output and daily weights
Calculate cumulative fluid balance and degree of fluid overload
Review medication list for potentially nephrotoxic medications and need for dosing adjustments based on GFR
Evaluate for underlying AKI cause/physiology
Treat hypotension, hypoperfusion, hypoalbuminemia, high abdominal pressure, and urinary tract obstruction
Evaluate nutrition and electrolyte composition of fluid intakes
Engage multidisciplinary team: pharmacy, dietitian, bedside nurses, family

GFR glomerular filtration rate, *AKI* acute kidney injury
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Medical Management of AKI Complications

Given the complexities of using KRT devices in small babies, awareness of available medical management options is an important part of care.

Electrolyte and Mineral Homeostasis

Neonates with AKI may be at risk for a variety of electrolyte abnormalities depending on the mechanism and severity of injury. For example, babies with gentamicin toxicity may maintain urine output but have elevated serum creatinine, hypokalemia, and hypomagnesemia secondary to tubular injury. Babies with severe oligo-anuric injury may develop hyponatremia secondary to fluid overload and/or severe hyperkalemia and hyperphosphatemia.

Hyperkalemia is a potentially life-threatening manifestation of AKI. In babies with oliguria and rising serum potassium levels, all fluids should be reviewed and adjusted for potassium content. Enteral feeds may need to be adjusted to lower-potassium formulations. Breast milk is an excellent choice due to low solute load and appropriate balance of nutrition. Potassium levels can be decreased also through urinary (e.g., with diuretics) or gastrointestinal excretion (e.g., with cation exchange resin), or via dialysis. Shifting potassium from the extracellular to intracellular compartments can be accomplished through use of base supplementation (sodium bicarbonate or acetate) to change pH, albuterol, and/or insulin and glucose. Serious adverse events including colonic necrosis and death have been described when cation exchange resins are given to babies directly [81]. To avoid direct administration, methods for pretreating infant formula with cation exchange resins have been published. Importantly, pretreating infant formula and breast milk alters the nutrient content, *decreasing* calcium and magnesium content and *increasing* sodium content [82, 83].

Infants with AKI may develop either hyponatremia or hypernatremia depending on the situation. Hyponatremia may develop as the result of total body fluid overload or as the result of sodium losses through chest tubes or gastrointestinal suction or through urinary sodium wasting in infants with congenital kidney and urinary tract disease (e.g., dysplasia, obstructive nephropathy). Fluid balance assessment and clinical context will guide appropriate management (fluid restriction vs. provision of sodium supplementation). Infants may also be at risk for hypernatremia as the result of large insensible losses (e.g., extremely premature infants with immature skin barrier), immature urinary concentrating ability, or large sodium intakes. Again, careful assessment of fluid balance and composition and volume of fluid inputs is important for determining appropriate response (e.g., provision of free water or increase in basal rate of fluids).

Infants with AKI may develop hyperphosphatemia due to impaired renal phosphorus excretion. Concentrated preterm formulas and human milk fortifier are especially high in calcium and phosphorus to promote bone health in premature infants as human milk is naturally low in all solutes [84]. Collaboration with a neonatology dietitian will allow for optimal adjustment of enteral nutrition if required for a more severe and extended episode of AKI. As with hyperkalemia, enteral nutrition may be pretreated with a phosphate-binding agent that decreases the phosphorus content of the feeds before they are given to the baby. Sevelamer is a noncalcium containing polymeric ion-exchange phosphate binder that has been studied in this setting [83, 85, 86]. As with ion exchange resins, pretreatment of enteral formula with sevelamer may decrease the calcium content. Calcium carbonate comes in a liquid formulation and is another enteral option.

Acid-Base Balance

Metabolic acidosis is a potential complication of neonatal AKI as the result of the underlying mechanism of the injury (e.g., large volume stool losses/diarrhea, poor systemic perfusion from sepsis or heart failure) or from impairment in excretion of acid as the result of decreased GFR. In addition, the immature tubular function of premature infants may result in urinary bicarbonate losses even without overlying AKI. Base supplementation may be required and can be given as either sodium bicarbonate or sodium acetate.

Therapeutics

Methylxanthines (Theophylline, Aminophylline, and Caffeine)

Theophylline and Aminophylline in Babies with Perinatal Asphyxia

Animal models and human studies demonstrate an increase in renal adenosine levels in states of acute renal hypoxemia and ischemia that then lead to renal vasoconstriction, decreased GFR, and a fall in urine output [87, 88]. These events serve as the rationale for the use of theophylline, an adenosine receptor antagonist, in the prevention of AKI in asphyxiated neonates. A single dose of theophylline (either 5 or 8 mg/kg) within 6 h of birth in these high-risk babies promotes improved urine output, lower serum creatinine rise, more favorable fluid balance, and less tubular injury [89]. The therapeutic effect appears to be most pronounced on days 3 and 5. All but one study of theophylline was completed in cohorts of term and postterm infants. Moreover, all were completed in centers without whole-body cooling. Aminophylline, a related salt of theophylline with the same active molecule, has also been evaluated in a small retrospective cohort of neonates who were cooled for moderate/severe HIE [90]. Treated infants who survived had improved urine output and decline in serum creatinine within 12 h of therapy, though the nonsurvivors in the treatment group did not show improvement in kidney function parameters.

Based on the currently available evidence, a single dose of theophylline within the first 6 h of life for newborns with perinatal asphyxia is endorsed in the KDIGO guidelines [91]. While the side effect profile of theophylline (seizures, arrhythmias, and gastrointestinal complications) is important, none of the above studies noted any difference in either mortality or adverse events between the treated and control groups. These findings should be confirmed in a larger, randomized controlled trial including in babies who are treated with whole body cooling.

Caffeine Use in Preterm Infants

Caffeine is an adenosine receptor antagonist that inhibits the preglomerular vasoconstrictive effects of adenosine and as such may be protective against AKI [92–94]. Two studies demonstrate AKI occurs less frequently in VLBW and preterm infants <33 weeks GA who received caffeine within the first week of life as compared to those who did not [93, 94]. In the first study by Carmody [93], the protective effect of caffeine against AKI appeared to be even stronger in the subgroup of babies who received prolonged mechanical ventilation. In a larger retrospective cohort (n = 675), Harer [94] showed babies who received caffeine had more AKI risk factors including lower average GA, lower birth weight, and higher severity of illness scores and were *still* less likely to develop stage 2 or 3 AKI. The role of caffeine in the mitigation of AKI in a cohort of neonates has also been documented in the setting of NEC with similar protective effects [92].

Diuretics

Diuretics are medications used to augment urine output in patients who have developed oliguria, though there is no evidence to support their use for the purpose of reversing the injury itself or improving outcomes [95]. They are widely used in the NICU, though with limited evidence to support their efficacy across clinical settings [96]. Awareness of their limitations as well as their side effects is important for rational and careful use of these medications. Loop diuretics act at the Na^+-K^+-$2Cl^-$ co-transporter in the thick ascending limb of the loop of Henle. Pharmacologic tolerance may occur with repeated doses as extracellular volume depletion associated with diuresis may trigger an increase in water and sodium reabsorption in other parts of the nephron (both proximal and distal tubules) [96, 97]. Side effects include hypotension, hypokalemia, and hypochloremic metabolic alkalosis as well as hypercalciuria that may lead to bone demineralization and nephrocalcinosis [98]. Ototoxicity is a rare complication that may occur with chronic use [99].

Thiazide diuretics act at the Na^+/Cl^- transporter in the distal tubule to promote water and sodium excretion. Thiazide diuretics are typically less potent than loop diuretics because less sodium is absorbed in this part of the nephron [96]. Unlike loop diuretics, thiazides promote calcium reabsorption in both the proximal and distal tubules [100] and may be protective against nephrocalcinosis.

Kidney Replacement Therapies (KRT)

Because of numerous technical challenges, the use of KRT in neonates historically has been considered a heroic intervention to be applied only when all other medical management has failed. Recent technological developments and the approval of extracorporeal blood purification devices designed specifically for babies have made KRT more feasible and will significantly alter the indications, timing of, modalities available for, and feasibility of KRT in these patients.

The use of peritoneal dialysis (PD), hemodialysis (HD), or extracorporeal continuous KRT (CKRT) depends on several factors including patient characteristics and hemodynamic stability, goals of therapy (fluid removal, electrolyte disorders, toxin removal), physician experience, and institutional resources. Each technique is associated with potential advantages and disadvantages; however, the optimal modality, dosing of, and timing for KRT initiation are unknown.

In general, the indications for KRT are the same in neonates as in older children. KRT should be applied to prevent further fluid accumulation, restore euvolemia, and metabolic balance, as well as to allow provision of adequate nutrition, blood products, and medications. Moreover, critically ill neonates with kidney dysfunction often have dysfunction in other organs as well. KRT can be initiated in situations where nonrenal conditions may lead to AKI, such as septic shock, cardiopulmonary bypass, inborn errors of metabolism, and hyperammonemia to improve outcomes.

Peritoneal Dialysis

PD has long been considered the modality of choice when treating neonates with AKI, including premature infants. It has a prominent role in the treatment of small infants after surgery for congenital heart defects [101]. Elective placement of a PD catheter at the time of surgery in infants determined to be at high risk for AKI is associated with shorter time to negative fluid balance and improved clinical outcomes as compared with age-matched infants undergoing similar surgery without PD catheter placement or use [102]. The popularity and success of PD for neonates is derived from its simplicity and effectiveness in even the smallest patients. This technique avoids the need for anticoagulation, provides excellent hemodynamic stability, and has a theoretical advantage in premature neonates related to the large peritoneal surface area-to-body ratio that provides improved dialysis efficacy. The lower dialysate fill volumes used for neonates generally necessitates the use of manual exchanges via commercially available two-chamber sets that allow inflow and outflow volumes to be measured with an accuracy of 1 ml. In this setting, dwell times are initially every 30–60 min provided continuously, a schedule that most often requires a one-to-one level of nursing support. To improve the efficacy of PD, some authors have suggested using continuous flow PD, but this requires two peritoneal catheters, or one double lumen catheter, instead of the usual one [103].

The most common issue in acute PD is the introduction of a suitable peritoneal catheter. With a need to start PD soon after catheter placement, there is an increased risk of catheter leak, infection, and mechanical complications [104, 105]. In term neonates, single-cuff straight (Tenckhoff®) catheters are available and are usually implanted with a paramedian entry-site. In some cases, the poorly represented peritoneal layer relative to the large caliber of the catheter and the internal cuff prevent a firm fixation of the catheter to the abdominal wall. In this case, fibrin glue (a mixture of fibrinogen and thrombin) can be applied on the external layer of the peritoneal membrane to produce a physiologic fibrin clot that may stop dialysate leakage, prevent infections, and eliminate the need for surgical interventions [106]. The fibrin clot then degrades over weeks by natural fibrinolysis.

Obtaining catheter access for PD is complicated in extremely or VLBW neonates by their small size and paucity of subcutaneous tissue. The distance between the umbilicus and the symphysis pubis in these patients is only 3–5 cm, complicating the placement of an infant straight Tenckhoff® catheter 30 or 31 cm long, with an intraperitoneal segment of 6–7 cm. In this setting, PD catheters are inserted percutaneously without tunneling under the skin, using a standard temporary PD catheter (7.5, 8.5, or 9 Fr, 8 cm length) or nontraditional catheters such as intravenous cannulas (22 or 24 gauge), central venous (14 gauge) or umbilical lines, angiocaths, and silicone wound drains. The lack of size-specific equipment for these patients and the use of improvised catheters are associated with PD malfunction due to leak and kinking [107].

Despite potential problems with access, PD is safe, simple, and inexpensive, thus representing the modality of choice for KRT in developing countries with limited resources. The main disadvantage of PD relative to extracorporeal blood purification therapies is the lower efficiency. HD and CKRT provide more rapid clearance of higher-molecular-weight solutes and faster fluid removal and are therefore superior for managing toxin removal, inborn errors of metabolism (IEM), and massive fluid overload (FO). Neonatal studies have reported a FO greater than 15–20% to be associated with poor outcomes including need for CKRT, prolonged NICU stay, time to extubation, or death within 30 days post-cardiothoracic surgery [108, 109]. In part because of these limitations in clearances and ultrafiltration, there has been some movement away from PD for the treatment of pediatric AKI in developed countries.

Hemodialysis

The use of intermittent HD for treating metabolic derangements in AKI or IEM is potentially associated with a superior solute clearance as compared with CKRT [110]. However, HD-associated hemodynamic instability and hypotension remain a significant problem for small patients. Animal data also suggest that high ammonia levels contribute to hypotension by up-regulating brain nitric oxide production that in turn suppresses the central sympathetic outflow causing systemic vasodilatation [111]. Given the strong limitations of HD due to its intermittent nature, complex application, high costs, and poor cardiovascular tolerance, the use of this technique in neonates with AKI has been supplanted by preference for CKRT.

Continuous Kidney Replacement Therapy

The use of CKRT in neonates was first described by Ronco in 1986 [112]. Four small newborns, ages 2–12 days, were treated with a modified continuous arteriovenous hemofiltration (CAVH) circuit. Blood lines were shortened to reduce the pressure drop, and a minifilter (0.005 m^2) was applied to optimize transmembrane pressure and ultrafiltration. Recently, the development of new machines for CKRT in neonates and small infants (either adapted or specifically conceived) has transformed infant extracorporeal blood purification from an orphan technology to an advanced care.

Technical challenges, including adequate vascular access, hypotension due to a relatively larger extracorporeal volume, and limited accuracy of fluid removal, have led to the preference of PD over CKRT in neonates with AKI. For example, a well-functioning vascular access using a central venous catheter can be difficult to obtain in very small infants, and the rates of both infectious and mechanical complications are exceedingly high. Access diameter significantly affects circuit survival. In the Prospective Pediatric CRRT (ppCRRT Registry) experience, none of the five French catheters used to provide CKRT in children with AKI lasted longer than 20 h [113]. Functional performances of catheters ranging from 7 to 9 French were better than with 5 Fr, with survival also favored by internal jugular vein insertion versus subclavian or femoral access. However, complications of catheterization are more common in babies because of the proportionally larger catheter caliber. Larger catheters relative to vessel size have the additional risk of causing central vessel stenosis, a complication that can impact the dialysis options of neonates and infants who will potentially have future KRT need. When using “standard” adult equipment with small central venous lines, access pressures surpass the machine operating limits with increased risk of alarms and shorter circuit lifespan. Machines designed specifically for small infants have overcome this problem by using a syringe-based system or miniaturized roller blood pumps. With appropriately sized equipment, single-lumen or small size dual-lumen catheters can be used in neonates to obtain adequate blood flows.

CKRT in neonates and infants requires a high precision of fluid balance and hemodynamic stability. In a case series of 174 children requiring CKRT, hypotension on connection was detected in 53 patients (30.4%) [114]. Extracorporeal circuit priming volume ideally should not exceed 10% of a patient’s circulating blood volume to avoid acute blood depletion with circuit initiation. When priming volume exceeds 10% of the patient’s circulating blood volume, a blood prime is strongly recommended to minimize hemodilution and hemodynamic instability. As an example, a 3 kg neonate requiring CKRT would have an overall blood volume of about 240 ml (80 ml/kg). Existing adult machines with pediatric set circuits have an extracorporeal prime volume in the range of 59–100 ml, thus exceeding the safe extracorporeal volume for neonates. This requires priming the circuits with blood, exposing the baby to potassium and citrate overload, transfusion reactions, infectious risk, and sensitization due to HLA exposure. Moreover, conventional HD and CKRT machines cannot control fluid balance better than ±30 ml/h and therefore are not approved for use with patients of low body weight (<8 kg).

Specialized Equipment

In 2005, Coulthard reported the results of an automated miniaturized syringe-driven machine to treat four babies weighing between 800 g and 3.4 kg, using a single-lumen access line, and without the need for blood-priming [115]. This device, the Newcastle Infant Dialysis and Ultrafiltration System (NIDUS®, Allmed, UK), is designed to provide single-lumen HD and ultrafiltration to children weighing between 800 g and 8 kg. A clinical study aimed at evaluating efficacy, safety, and outcomes of NIDUS machine as compared with the other existing KRT methods for babies <8 kg is currently active in UK.

In 2014, Ronco published the first-in-human use of a miniaturized Cardio-Renal Pediatric Dialysis Emergency Machine (CARPEDIEM®, Bellco-Medtronic, Mirandola, Italy) for treating a critically ill neonate weighing 2.9 kg with multiorgan failure and severe fluid overload [116, 117]. This device is a pump-driven machine that functions with a small double lumen catheter, and it is designed to provide multiple blood purification therapies to infants 2.5–10 kg including both pre- and postdilution CVVH as well as therapeutic plasma exchange and co-current CVVHD.

In 2016, Askenazi described the adaptation of an ultrafiltration device designed for fluid removal in adults with diuretic-resistant heart failure (Aquadex FlexFlow System®, CHF Solutions Inc., Eden Prairie, MN, USA) to provide a range of therapies, including CVVH, prolonged intermittent KRT, and slow continuous ultrafiltration, to children with AKI [118]. KRT with this device resulted in minimal complications in critically ill neonates and more than 50% of infants weighing <10 kg survived to come off KRT or transition to another modality of kidney support [119].

Novel technology specifically dedicated for neonates and small infants will further facilitate the application of CKRT in such patients. Operating characteristics of these machines are detailed in Table 5. The possibility to treat neonates with simplified and safe methods will likely induce care teams to apply extracorporeal blood purification earlier and for extended indications. CKRT can now be used in neonates not only in the case of extreme clinical conditions (i.e., rescue therapy for patients with severe fluid overload and peritoneal dialysis failure), but also for other indications requiring timely CKRT start, such as fluid balance control, hyperammonemia, drug intoxication, and short transient oliguric states with hypercatabolic complications.

Kidney Replacement Therapies in Inborn Errors of Metabolism

Inborn errors of metabolism (IEM) are a class of genetic disorders associated with failure of the metabolic pathways involved in either the breakdown or storage of carbohydrates, fatty acids, and proteins. Hyperammonemia (>100 umol/L or 170 ug/dL in neonates) is among the most important laboratory findings associated with IEM and is mainly due to urea cycle disorders or organic acidemias. This condition requires prompt recognition and aggressive therapy aimed at minimizing endogenous production and promoting removal of ammonia. The continued expansion of newborn screening allows for early identification and treatment, leading to reduced morbidity and mortality. However, early onset acute hyperammonemia can still present in neonates within the first few days of life after they start to feed, potentially resulting in acute encephalopathy. KRT are a cornerstone in the treatment of this condition, especially in those patients who have a rapid rise of serum ammonia levels (>500 μmol/l or 852 μg/dl) or fail to respond after 4 h of initial medical and nutritional management. Adverse prognostic factors include hyperammonemic coma lasting >3 days, increased intracranial pressure, and/or a plasma ammonia peak level >1.000 μmol/l (1.703 μg/dl) [120].

Intermittent HD and CKRT have proved more efficacious than PD in reducing ammonia levels. However, in an Italian multicenter study, delayed extracorporeal treatment was not superior to PD in improving the short-term outcome of neonates with hyperammonemia [121]. Predialysis ammonia levels, but not dialysis modality, were associated with death and neurologic sequelae. Thus, an aggressive and expeditious management of these patients is needed, one in which the choice of KRT modality should take into consideration the local resources, condition of the patient, serum ammonia levels trends, response to nitrogen-scavenger therapy, and patient's body weight. In general, PD should be performed in situations where extracorporeal therapies are not available or are unsafe, while extracorporeal therapies should be used in neonates who show a rapid rise in blood ammonia levels. Overall, with serum levels of ammonia in the range of 300–500 μmol/l (513–850 μg/dl) and while on scavenger treatment, the priority is providing the small patients with adequate dialysis access. Detailed

Table 5 Characteristics of extracorporeal dialysis machines specifically designed or adapted to treat neonates and small infants

Monitor	Modalities	Priming volume (ml)	Filter surface (m^2)	Membrane	Qb range (ml/min)	Qr or Qd range (ml/h)	UF rate range (ml/h)
NIDUS® (syringe-based system)	Single-lumen HD and ultrafiltration	5 + 4 to 10 ml of stroke volume	0.045	Polysulfone	Stroke volumes of 5 ml/kg up to 2 kg body weight, and then 10–12 ml	0–400	0–60 (±1)
CARPEDIEM®	CVVH CVVHD PEX	27 33 41	0.075 0.15 0.25	Polyethersulfone	2–50 (±1)	10–600	0–40 (±1)
Aquadex FlexFlow System® (adapted)	CVVH SCUF	33	0.12	Polysulfone	10–40 (±5)	External in-line medication infusion machine	0–500 (±10)

HD hemodialysis, *ml/kg* milliliters per kilogram, *ml* milliliter, *m* meters squared, *Qb* blood flow, *Qr* replacement fluid flow, *Qd* dialysate flow, *UF* ultrafiltration, *CVVH* continuous veno-venous hemofiltration, *CVVHD* continuous veno-venous hemodiafiltration, *PEX* plasma exchange, *SCUF* slow continuous ultrafiltration consensus guidelines for management of hyperammonemia in pediatric patients receiving CKRT have been published [122].

Distant Organ Effects

AKI as a syndrome has effects beyond the kidneys and the acute period. Emerging evidence highlights the relationship between AKI and distant organs in neonates including the brain and lungs. AKI has been associated with an increased likelihood of intraventricular hemorrhage (IVH) [123] as well as worse outcomes (death and disability) in babies with perinatal asphyxia [36]. AKI also likely has an association with lung disease and pulmonary outcomes. Recent work shows near-term/term babies with AKI have increased odds of chronic lung disease or death as well as longer duration of need for respiratory support [124]. Similarly, premature infants 29–32 weeks gestational age with AKI had higher odds of developing moderate/severe bronchopulmonary disease or death [125]. Inflammation is an underlying pathway for all these processes. Additional investigation into organ crosstalk will certainly benefit short- and long-term outcomes and highlights the importance of ongoing collaboration between neonatologists and nephrologists in the care of these fragile patients.

Long-Term Outcomes and Future Directions

The field of neonatal AKI has seen tremendous advances over the last decade in terms of our understanding of the AKI syndrome in both preterm and term infants with a range of underlying conditions and comorbidities. The ability to support these patients both medically and with KRT has also seen major advancements. Improved AKI survival for neonatal patients overall entails the need to attend to long-term kidney outcomes for these patients. CKD has been documented in AKI survivors of several conditions including TTTS and congenital heart disease [29, 62]. The long-

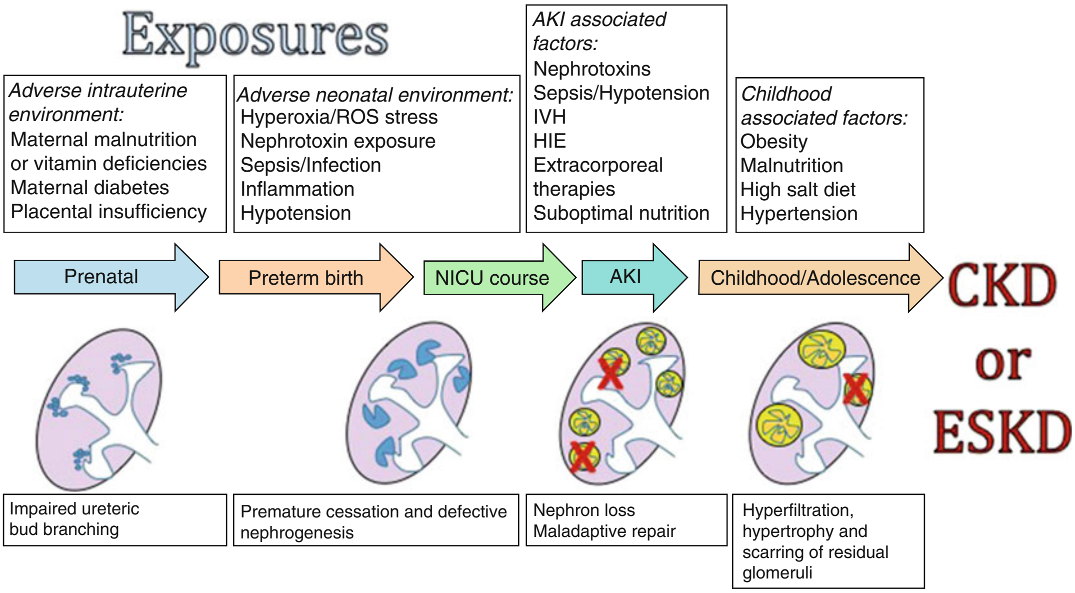
Exposures
Adverse intrauterine environment:
Maternal malnutrition or vitamin deficiencies
Maternal diabetes
Placental insufficiency
Adverse neonatal environment:
Hyperoxia/ROS stress
Nephrotoxin exposure
Sepsis/Infection
Inflammation
Hypotension
AKI associated factors:
Nephrotoxins
Sepsis/Hypotension
IVH
HIE
Extracorporeal therapies
Suboptimal nutrition
Childhood associated factors:
Obesity
Malnutrition
High salt diet
Hypertension
Prenatal
Preterm birth
NICU course
AKI
Childhood/Adolescence
CKD or ESKD
Impaired ureteric bud branching
Premature cessation and defective nephrogenesis
Nephron loss
Maladaptive repair
Hyperfiltration, hypertrophy and scarring of residual glomeruli

Fig. 1 Exposures across the life course lead to an increased risk of chronic kidney disease in preterm infants. Legend: Preterm infants have many exposures across their life course that predispose them to both acute kidney injury and chronic kidney disease. Awareness of the potential insults at every step may improve our ability to monitor for and mitigate these events in order to optimize kidney and overall health as these children grow. (© Reprinted with permission from Springer Nature Publishing, *Journal of Perinatology*, Harer et al. [131])

term kidney risks for premature infants related to their reduced nephron endowment have been described in several registry cohort studies [126–128] as well as some smaller, single-center retrospective cohorts [129, 130]. The attributable risk of AKI for these patients has yet to be explored prospectively and is one of the many exposures across the lifespan that put preterm infants at long-term risk for CKD (see Fig. 1) [131]. Evidence-based guidelines for appropriate follow-up of these patients are also lacking. Ongoing collaboration between neonatologists and nephrologists is critical for improving kidney function monitoring, AKI documentation, and AKI mitigation strategies whenever possible and thereby reducing chronic health risks as these children grow.

Cross-References

- ► Infant Dialysis
- ► Pathogenesis of Acute Kidney Injury
- ► Peritoneal Dialysis

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Evaluation and Management of Acute Kidney Injury in Children

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Abstract

Acute kidney injury (AKI) occurs in 5% of hospitalized children and 20–30% of children admitted to the intensive care unit (ICU). AKI is associated with poor patient outcomes, including increased risk of mortality, prolonged length of ICU and hospital stay, and prolonged invasive mechanical ventilation. Children with severe AKI and those requiring kidney replacement therapy (KRT), particularly infants and those with multiple organ dysfunction, experience mortality rates up to 70%. AKI has now been acknowledged as a common and frequently overlooked multidimensional syndrome, with additional long-lasting negative impacts on kidney health, even when function has apparently recovered after the inciting episode. The present chapter will extensively discuss how to identify and accurately diagnose an AKI episode in a timely fashion, novel research tools that are currently available to anticipate an AKI episode, risk factors that the clinician should be aware of to prevent kidney injury, and available pharmacological strategies, including management of fluid balance. An extensive section detailing technical and relevant aspects of acute pediatric dialysis is provided at the end of the chapter.

Current initiatives to guide the care of patients with AKI and focus on increasing awareness (by patients, healthcare providers, and healthcare systems) as well as on research to improve AKI diagnosis and healthcare delivery, with a goal of improving the currently poor outcomes in this particularly at-risk population, are discussed.

Keywords

Renal replacement therapy · Acute kidney injury · Continuous renal replacement therapy · Fluid overload · Nephrotoxic medication

Introduction

Acute kidney injury (AKI) occurs in 5% of hospitalized children and 20–30% of children admitted to the intensive care unit (ICU). In the last 15 years, a large volume of literature has elucidated the potent negative impact of pediatric AKI on short- and long-term patient outcomes, highlighting the burden to patients and healthcare systems. Although proven pharmacological therapies for AKI still elude us and the approach to supporting children with severe acute kidney dysfunction, kidney replacement therapy (KRT), has not changed substantially, there has been an evolving importance placed on early identification and prevention and/or mitigation of AKI

Table 1 The kidney disease: improving global outcomes (KDIGO) definition of pediatric acute kidney injury

Stage (severity)[a,b]	Serum creatinine (SCr) criteria	Urine output criteria
1	SCr rise to 1.5 to <2 times baseline SCr within a 7 day period OR ≥ 0.3 mg/dL (26.5 μmol/L) SCr increase from baseline SCr within a 48 h period	<0.5 ml/kg/h for 6–12 h
2	SCr rise to 2 to <3 times baseline SCr	<0.5 ml/kg/h for ≥ 12 h
3	SCr rise to ≥ 3 times baseline SCr OR Increase in SCr to ≥ 4 mg/dL (353.6 μmol/L) OR Initiation of renal replacement therapy for acute kidney injury OR Decrease in estimated glomerular filtration rate to <35 ml/min/1.73 m[b] (using equation as described in text)	<0.3 ml/kg/h for ≥ 24 h OR Anuria for ≥ 12 h

[a] Patients fulfill criteria for AKI if they attain either the SCr *or* the urine output criteria
[b] The cause of AKI should be identified

and its complications. This chapter will review standard clinical evaluation and differential diagnosis of AKI, while considering ongoing novel research in diagnostic AKI biomarkers, risk stratification, and quality improvement in the context of existing guidelines [1–3], followed by a clinically practical approach to supporting patients with severe AKI using KRT. The focus of this chapter is on non-neonatal patients; a dedicated chapter will address neonatal AKI.

Definition

One of the most important research advancements in the AKI field has been the development of a standardized, multidimensional AKI definition, which led to an exponential increase in research literature describing pediatric AKI incidence and outcomes [4–8].

The Kidney Disease: Improving Global Outcomes (KDIGO) AKI Definition

In 2004, the first contemporary AKI definition was developed by an international consensus group, the Acute Dialysis Quality Initiative (ADQI) [9]. This definition evolved via several iterative changes specific to children and adults, to the currently accepted, harmonized child-adult definition, the 2012 KDIGO classification [1] (Table 1). The underlying concept is to employ changes in current biomarkers of kidney function (*increase* in serum creatinine [SCr] *or decrease* in urine output), to define AKI, and to stratify AKI severity. For example, as shown in Table 1, a child may be diagnosed with AKI if they fulfill criteria for acute SCr rise from baseline (based on a 48 h or 7 days time frame) *or* if they develop hourly urine output below a given threshold. Furthermore, depending on how high SCr rises or on how low and for how long urine output decreases, AKI severity is staged as being mild (Stage 1) or severe (Stage 2 or 3). Recently, AKI duration has been suggested as a marker of AKI severity indicated by the KDIGO classification. In this line, transient AKI (lasting less than 48 h before restoration of baseline kidney function) and persistent AKI (lasting more than 48 h) have been identified as further aspects related to the severity of the disease and associated with clinical outcomes [10].

Challenges with AKI Definition

Certain inherent limitations must be acknowledged about the current KDIGO AKI definition. First, SCr is not an ideal marker of glomerular filtration rate (GFR) for many reasons [11]. Serum creatinine concentrations are affected by muscle mass, which not only changes with age, but may also be altered by changes in nutritional status, chronic illness, or prolonged ICU stay. As GFR decreases acutely, SCr secretion by the kidney tubules increases, which may contribute to reduction in AKI ascertainment. In addition, SCr is affected by volume status, where significant volume overload can dilute SCr concentrations. This phenomenon may potentially mask SCr increase and thus also lead to underappreciation of AKI occurrence or severity. For example, imagine a patient who is postoperative cardiac surgery and receives large amounts of fluid for resuscitation, and in whom SCr concentration rises by 10 μmol/l (or ~0.11 mg/dl) from a baseline SCr concentration of 30 μmol/l (0.34 mg/dl); it is likely that the patient's SCr concentration, after aggressive fluid resuscitation, is diluted. Therefore, the true extent to which SCr has risen from baseline would be underappreciated. It has been proposed to correct SCr values for the extent of positive fluid balance, in order to appreciate what the patient's true SCr would be in the euvolemic state [12]. Simply put, the patient's SCr on a given intensive care unit day can be multiplied by a factor, which is a ratio of their fluid balance to their total body water:

Adjusted SCr = measured SCr × (1 + (fluid balance in liters from ICU admission/total body water), where total body water (liters) may be estimated as body weight × 0.6 in older children to as high as 0.8 in neonates.

The adjusted SCr may then be used to assess for AKI. AKI ascertained using this method was shown to lead to a stronger association of AKI with poor outcomes in adults and children [12, 13]. While it may seem cumbersome to perform such calculations in clinical practice, consideration of not only SCr concentration rise but also the patient's acute change in volume status may help in evaluating AKI severity in some patients.

Another potential problem in interpreting and applying SCr-based AKI definitions is the need to evaluate acute SCr rise from *baseline*; however, a child's baseline SCr is often unknown. A falsely low estimate of baseline SCr will result in overestimation of AKI. Conversely, when a more conservative or higher estimate of baseline SCr is used, some cases of mild AKI may be missed, or the presence of more severe AKI may be incorrectly assumed to be mild. To address the problem of unavailable baseline SCr values, it is recommended to estimate baseline SCr by assuming that the patient has a normal baseline GFR. In the previously healthy child, baseline (eGFR) may be estimated as 120 ml/min/1.73 m^2. Using the most recently proposed eGFR equation (GFR = 36.5 × (height in cm/SCr in μmol/l) or 0.413 mg/dl × (height in cm/SCr in mg/dl)) [14], the estimated baseline SCr may be imputed from this baseline eGFR (i.e., estimated baseline SCr = 36.5 × (height in cm/120 ml/min/1.73 m^2). In cases where height is unavailable, either a height-independent eGFR equation may be used or normative SCr values for age and sex from local laboratories may be used to estimate baseline SCr [15, 16]. Another baseline SCr challenge is deciding *which* previously measured SCr should be considered as the patient's baseline for a current AKI episode. Use of the lowest SCr in the prior 3–6 months makes widespread application of AKI definition in the research setting reasonably feasible. However, in the clinical setting, it may be better to use a baseline SCr that is more relevant for a given patient within a given scenario. For example, if 2 months prior to the current AKI episode, a patient was admitted to hospital with severe failure to thrive and a documented very low SCr value during that hospitalization, but more recently had a higher outpatient SCr value when clinically well (perhaps better nourished, higher muscle mass), it may be reasonable to utilize the more recent SCr concentration to represent this patient's baseline kidney function. In other words, in the clinical setting, it is worthwhile to take a moment and consider what the best estimate of the patient's baseline kidney function is, given their individual history and clinical characteristics.

There are also inherent challenges to the KDIGO urine output criteria. These criteria assume that urine output is being accurately and consistently measured (e.g., Stage 1 AKI is <0.5 ml/kg/h for 6–12 h). Different ICUs have varying practices in the use of indwelling bladder catheter and in recording of urine output on an hourly or quasi-hourly basis. Therefore, in order to avoid missing AKI by urine output criteria, it is important to identify patients who require more accurate urine output monitoring and to ensure this is done. Moreover, urine output rate must also be evaluated in the clinical context of hourly fluid administration to the patient. On noncritical care wards, the use of urine output criteria is clearly limited; but again, in patients who are at high risk for AKI, accurate urine output monitoring should be prioritized [3]. Despite the challenges in applying the urine output KDIGO criteria, they are important to consider as up to two-thirds of patients who fulfill urine output criteria of AKI diagnosis may be missed if only the SCr criteria are evaluated [7].

Another challenge with the KDIGO AKI definition is that it may not be as useful in all clinical contexts; they were developed for use in the critical care setting. However, not all causes of AKI will present with SCr rise or urine output decrease as suggested by this definition. For example, severe AKI due to cisplatin nephrotoxicity or other nephrotoxins which lead to urinary concentration defects will unlikely present with oliguria. In the case of cisplatin nephrotoxicity, for example, it is quite routine for patients to receive large amounts of intravenous fluids, which makes interpretation of change in SCr difficult.

What the issues above highlight is that in clinical care (as opposed to research) the KDIGO AKI definition should be used as a tool which is interpreted in the specific clinical context of the patient. It is imperative to consider each patient's history, and physical and laboratory examinations when deciding whether a patient has AKI and how severe it is, and not to base clinical decisions solely on KDIGO AKI staging. For example, while a patient may only have Stage 1 KDIGO AKI (e.g., 50% SCr rise from baseline), if they show other signs of more severe kidney injury (e.g., hyperphosphatemia, fluid overload, hyperkalemia), it would be inappropriate to make management decisions (e.g., allowing liberal fluid intake) based exclusively on the presence of Stage 1 AKI. The converse is true in cases of more severe AKI according to the KDIGO definition, but with no other evidence of AKI or risk for AKI using other clinical methods.

Epidemiology

Incidence, Disease Patterns, and Risk Factors

The incidence of AKI has been most extensively studied in critically ill children. Acute kidney injury incidence estimates range from 15 to 70% in children undergoing cardiac surgery [8, 17, 18] and 8 to 27% in noncardiac critically ill children [7, 19–21], dependent upon characteristics of patients studied. In the multinational AWARE study (Assessment of Worldwide Acute Kidney Injury, Renal Angina and Epidemiology study) of over 4000 ICU children with a predicted ICU length of stay of ≥48 h, AKI occurred in 27% of patients. Approximately half of patients with AKI developed Stage 2 or worse AKI (i.e., doubling of SCr from baseline), consistent with previous smaller studies. A preliminary analysis of a 26-center (North America and Europe) study including patients admitted ICU with confirmed severe acute respiratory syndrome coronavirus 2–positive diagnosis during the 2020 pandemic (106 children, ~45% with AKI) also showed that about half of patients with AKI developed Stage 2 or worse AKI severity [22].

Consistently, across all studies, increasing patient illness severity increases the risk of AKI. For example, in children treated with extracorporeal membrane oxygenation (ECMO), incidence of AKI approaches 75–90% [23, 24]. In the cardiac surgery setting, young age, cardiopulmonary bypass duration, and surgical complexity are important factors for AKI [25]. In noncardiac surgery ICU patients, AKI risk factors have included various markers of illness severity (e.g., vasopressors, higher illness severity/mortality scores): use of nephrotoxic medications, younger age, and oncologic diagnoses [7, 20, 21, 26]. In cardiac surgery and noncardiac surgery patients, AKI occurs early, with most AKI becoming apparent within 72–96 h of admission [19, 20, 25, 27]. Moreover, patients who have even Stage 1 AKI at the time of ICU admission are much more likely to develop more severe AKI later in admission, versus patients with no AKI at admission [7]. One two-center study showed that almost

10% of patients with AKI in the ICU do not have complete resolution of AKI (i.e., still fulfill criteria for AKI) by hospital discharge and that lack of recovery from AKI occurs more commonly in patients who develop Stage 3 AKI [28].

In non-intensive care unit patients, AKI incidence is less clear. In hospital-based studies, 5–30% of non-ICU pediatric admissions were associated with AKI; the variability in AKI rate was dependent on the frequency of SCr monitoring [29–31]. Children treated for cancer, especially with stem cell transplant, have been fairly well characterized for AKI development [32]. Most studies evaluating these patients have used SCr doubling from baseline as the AKI definition, which occurs in approximately 20% of patients after stem cell transplantation. The timing of AKI appearance in these patients is different from ICU patients, since throughout chemotherapy treatment for solid tumors and for several months after stem cell transplant, these children are exposed to repeated risk episodes for AKI from infection, radiation treatment, thrombotic microangiopathy, use of nephrotoxic medication, and intravascular volume depletion. Therefore, AKI may occur in the weeks to months after the initial hospital admission.

Nephrotoxic medication exposure is common in the hospital setting. Importantly, nephrotoxic medication exposure also represents a *modifiable* AKI risk factor and a potentially important opportunity for AKI prevention strategies. More than 80% of hospitalized children are exposed to at least one nephrotoxic medication during admission [33]. Approximately 12–30% of non-ICU children treated with aminoglycoside antibiotics develop AKI during treatment [34, 35]. In a large, single center study, 25% of children receiving aminoglycosides for at least 3 days *or* ≥ 3 nephrotoxins simultaneously developed AKI [36]. Another study demonstrated that increasing nephrotoxic medication exposure was associated with a concomitant increase in AKI risk [33]. Non-steroidal anti-inflammatory drugs have been associated with a substantial AKI rate in non-ICU hospitalized children [37]. Nephrotoxic medication associated AKI is covered extensively a dedicated chapter.

Short-Term Outcomes

A large body of literature has demonstrated that AKI is independently associated with poor patient outcomes, including increased risk of mortality, prolonged length of ICU and hospital stay, and prolonged invasive mechanical ventilation [7, 17, 21]. Children with AKI requiring KRT, particularly infants and those with multiple organ dysfunction, demonstrate mortality rates ranging from 30 to 70% [38]. However, even KDIGO Stage 1 and 2 AKI have been shown to be associated with mortality, independent of illness severity [19, 20, 27]. Two recent large studies (the AWARE study described above and a Chinese study), and several previous smaller studies in different ICU specific patient populations, have shown that AKI Stages 2 or 3 are strongly and consistently associated with ICU and hospital outcomes [7, 31]. In the non-KRT-requiring AKI setting, the mechanisms for associations with poor patient outcomes remain unclear; the less severe AKI stages do not typically cause life-threatening sequelae like hyperkalemia. One proposed pathophysiologic effect of AKI on outcomes is from evidence of organ injury “cross-talk” (cardio-renal or pulmonary-renal interactions) [39, 40]. Animal studies suggest this organ crosstalk is mediated by pro-inflammatory cytokines [41]; this is supported by data in humans, where patients with AKI have massive inflammatory mediator activation compared to healthy controls [42]. While reduction in these cytokines prevents AKI in animal models, a similar AKI prevention strategy has not been evaluated in humans to date. There is a large literature base showing that fluid overload is strongly associated with mortality and morbidity in patients with AKI who and who do not receive KRT [24, 43–47]. Thus, early treatment or prevention of fluid overload may be a modifiable, outcome-mitigating AKI complication.

Long-Term Outcomes

Animal models suggest that the link between AKI and chronic kidney disease (CKD) includes molecular or cellular triggers of chronic fibrosis and permanent kidney mass loss causing hyperfiltration mediated damage [48, 49]. In adults, even Stage 1 AKI is a risk factor for long-term CKD and mortality [50–53]. Evaluating this association in children has been very challenging because of the lack of existing large, administrative databases with post-discharge kidney function measures and because screening for CKD after an AKI episode in children has not been the standard of care. As such, AKI represents a missed opportunity to follow patients who may be at-risk for CKD development [54]. Several past single center pediatric studies, only including follow-up of AKI patients, have shown a higher incidence of long-term CKD and hypertension in children with AKI, relative to the general pediatric population. Recently, a few observational studies including AKI *and* non-AKI patients have assessed the extent to which AKI is associated with later development of CKD, hypertension, and other patient outcomes. In a retrospective two-center cohort of about 2500 ICU children, AKI during ICU admission was associated with mortality, increased healthcare utilization, CKD, and hypertension diagnosis (defined using diagnosis codes), in the 5 years after hospital discharge [4–6, 26]. A prospective two-center study of approximately 250 children showed that Stage 2 or worse AKI during ICU admission was associated with a sixfold increased risk of CKD or pre-hypertension at 6-year follow-up [55]. In non-ICU hospitalized children, one study showed that 70% of children exposed to nephrotoxins had evidence of CKD at 6-months follow-up [56]. Literature on long-term kidney outcomes of AKI after cardiac surgery is controversial. Two multicenter studies of children undergoing cardiac surgery showed that these patients have a high prevalence of hypertension and CKD (approximately 15–20%) at 4 to 5 years after surgery, but that postoperative AKI was not associated with these outcomes [57, 58]. A retrospective study conversely showed that 5-year post-cardiac surgery CKD incidence was 12% vs. 3% in patients with *vs.* without AKI [59]. Regardless of the uncertain results on the long-term impact of AKI, children having cardiac surgery are at high risk for long-term kidney disease and hypertension [60]. In children undergoing stem cell transplant, AKI was associated with CKD and hypertension up to 30 years later [61]. Although more research is needed, the data available thus far provide some information for families and justify considering post-discharge kidney follow-up in patients who develop AKI, especially Stage 2 AKI or worse.

Etiology and Approach to Evaluation

General Concepts and History-Taking

In the critical care setting, identification of a single cause of AKI is unusual as AKI is often multifactorial. In the noncritical care setting, such as the emergency room or hospital wards, it is more likely to find a single important etiology, such as nephrotoxic medication use in the patient treated for a cystic fibrosis exacerbation or a patient presenting to the emergency room with gross hematuria and hypertension, suggesting acute glomerulonephritis. While it is important to remember that most AKI occurring in hospitalized patients is due to acute tubular necrosis (ATN), it is also important to remember that ATN is a pathological diagnosis which has many causes, including severe hypoperfusion, nephrotoxic medication use, sepsis, and rhabdomyolysis [62]. Systematic identification of all potential AKI causes is crucial to provide appropriate, focused recommendations for diagnostic evaluation and avoidance of further kidney injury. Moreover, during the initial AKI evaluation, it is always important to consider and exclude, whether by history or laboratory results, causes that require immediate therapeutic intervention (e.g., rapidly progressive glomerulonephritis; hemolytic-uremic syndrome; acute interstitial nephritis, rhabdomyolysis; severe nephrotoxicity; acute obstruction; renovascular thrombosis). The overall goal of AKI evaluation should be to seek out such causes and risk factors, while ruling out less common causes of AKI.

Despite the fact that AKI is often multifactorial in nature, a useful framework for approaching AKI evaluation has been to separate AKI etiologies into three major categories: *decreased kidney perfusion* (for simplicity, renovascular events are included in this category), *intrinsic causes* (comprising a variety of kidney tissue diseases and ATN of different causes) and post-renal causes (primarily, obstruction), shown in Table 2.

The category of decreased kidney perfusion includes conditions wherein only function is altered (e.g., vomiting and intravascular volume depletion with SCr rise due to adaptive decrease in GFR, which is *fluid-responsive*) as well as conditions wherein kidney tissue may or may not be damaged (e.g., low cardiac output syndrome, nephrotic syndrome, hepatorenal syndrome, which is not *fluid responsive*; Table 2). In other words, not all causes of decreased kidney perfusion should be thought of as fluid-manageable. Decreased kidney perfusion occurs commonly in the ICU and in patients undergoing stem-cell transplants, resulting from excessive blood loss, sepsis with capillary leak, cardiac dysfunction, vomiting, inadequate fluid replacement, and burns. A detailed fluid balance history (looking for negative fluid balance) and examination for weight loss will help reveal the extent to which fluid losses may be contributing to the AKI episode and to which fluid resuscitation will benefit. A large positive fluid balance or progressive weight gain (described in more detail below) may suggest more severe AKI or point to causes associated with edema and decreased kidney perfusion (e.g., heart failure, nephrotic syndrome, hepatorenal syndrome) or of fluid redistribution from the intravascular space (e.g., capillary leak). In some cases of decreased kidney perfusion associated with volume overload, such as with nephrotic syndrome or hepatorenal syndrome, albumin infusions may help redistribute body fluid into the intravascular space, increase kidney perfusion and delivery of loop diuretics to the Loop of Henle. The category of intrinsic AKI denotes that there is damage to the kidney parenchyma or tubular, glomerular, interstitial, and vascular tissue. Intrinsic AKI thus also includes ATN which may occur due to severe decreased kidney perfusion. Other causes of intrinsic AKI (e.g., glomerulonephritis, typically associated with hypertension and hematuria) are discussed in other chapters; however, it is important to elicit any systemic symptoms (e.g., arthritis, pulmonary hemorrhage, rash, photosensitivity) which may provide insight into specific diagnoses. It is important to consider acute interstitial nephritis, which may or may not develop after exposure to an offending agent (e.g., antibiotics, proton pump inhibitors, NSAID). A detailed medication history will elucidate whether medication-induced nephrotoxicity (e.g., aminoglycosides, amphotericin-B, chemotherapeutic agents, angiotensin converting enzyme inhibitors, calcineurin inhibitors,

Table 2 A simple classification for causes of AKI, with some examples of causes

Decreased kidney perfusion	Intrinsic	Post-renal
Hypotension or reduced intravascular volume Dehydration/hypovolemic shock due to GI, urinary, or other losses Hemorrhagic shock Sepsis *Reduced colloid pressure* Nephrotic syndrome Liver failure Malnutrition/hypoalbuminemia Sepsis/capillary leak Burns *Reduced cardiac output* Cardiogenic shock due to heart failure, tamponade, etc. *Vascular* Renal vein stenosis/thrombosis Renal artery stenosis/thrombosis Malignancy with secondary mass effects on vessels	*Glomerular* Glomerulonephritis *Tubular (includes ATN)* Hypoxic-ischemic injury Hemolysis Rhabdomyolysis Tumor lysis syndrome Toxins *Interstitial* Pyelonephritis Acute interstitial nephritis *Vascular* Hemolytic uremic syndrome Thrombosis or stenosis of renal vessels (artery or vein) Malignant hypertension	*Renal obstruction with solitary kidney* Ureteral-pelvic junction Ureteral stenosis Ureteral-vesical junction Malignancy with secondary mass effects *Bilateral ureteral obstruction* Stones Malignancy *Urethral obstruction* Posterior urethral valves Catheter obstruction

radiocontrast agents, discussed in a dedicated chapter) may be contributing to the current AKI episode. Non-medication nephrotoxic causes include myoglobin (i.e., rhabdomyolysis) and plasma free-hemoglobin (e.g., with severe hemolytic anemia or during ECMO).

Physical Examination

The physical exam may provide evidence of the cause of AKI. A rash or arthritis may suggest vasculitis, whereas ascites or jaundice may suggest hepatorenal syndrome. Low blood pressure and poor perfusion will strongly suggest ischemic ATN. The physical exam also helps to evaluate the severity of fluid overload associated with AKI. Correlating changes in oxygen needs with changes in fluid balance may help determine the extent to which fluid overload is impeding adequate oxygenation and ventilation [43]. Careful attention to magnitude of fluid overload is mandatory in patients with AKI due to its prognostic significance (discussed above) and implications on management. Different methods for fluid overload assessment based on calculating changes in daily weights and fluid balance as a percentage of estimated dry weight have been proposed [24, 63, 64]. For critically ill patients, the most commonly reported formula uses the difference in fluid output from fluid intake since ICU admission, as a percentage of the patient's estimated dry weight, using the following formula to quantify the degree of fluid overload:

Fluid overload (%) = (fluid in liters – fluid out liters)/weight in kg * 100% [64].

In units which perform daily weight measures, an alternative weight-based formula for calculating fluid overload may be used:

Fluid overload (%) = [*current weight (kg) – ICU admit weight (kg)*]/*admit weight (kg) * 100*

Fluid overload greater than 10–15% has been associated with poor outcomes in critically ill children [65]. Worsening of fluid overload, despite attempts at diuresis, may imply the impending need for KRT.

Laboratory Investigations

Laboratory investigation can help elucidate specific AKI causes as well as evaluate its severity. Table 3 shows suggested laboratory investigations in AKI. Urine examination assesses for evidence of glomerulonephritis (hematuria, red blood cell casts, white blood cell casts), interstitial nephritis (pyuria, urine eosinophils), or renovascular thrombosis (e.g., isolated hematuria without casts, in the presence of hypertension). Patients with ATN may have granular or muddy brown casts. Other laboratory investigations listed in Table 3 are helpful to assess for other concurrent causes of AKI. For example, the complete blood count and smear are essential to assess for thrombotic microangiopathy (low hemoglobin, thrombocytopenia, and schistocytes). A kidney ultrasound is often performed for first AKI evaluation, to evaluate anatomy and determine if obstruction or renovascular thrombosis is present using Doppler imaging evaluation. With ATN, the kidneys may appear normal on ultrasound examination, or may be enlarged and/or hyperechogenic with or without elevated resistive indices on Doppler interrogation. Kidney biopsy is typically reserved for patients in whom acute intrinsic renal diseases other than ATN may be suspected (e.g., glomerulopathies due to different causes, presenting with significant proteinuria and/or hematuria; acute interstitial nephritis presenting with severe hypertension, pyuria, and consistent clinical history; patients presenting with a suspected systemic disease known to be associated with kidney disease, such as lupus). On kidney biopsy, ATN may be associated with different histopathological findings depending on severity and cause of ATN (reviewed in detail in the following reference) [66]. In general, the glomerular compartment is relatively spared and injury is evident in the tubular compartment and potentially the interstitium. Histopathological evidence of tubular injury may be quite patchy or may appear quite subtle, especially with ischemic ATN; with direct tubular toxicity, the tubular epithelial cell changes

Table 3 Laboratory Investigations for AKI

Urine Testing
Urinalysis and Urine Culture Dipstick testing for hematuria, proteinuria, signs of infection/inflammation (leukocytes, nitrites) or of interstitial nephritis (leukocytes) Urine culture should be collected by catheterization (non-toilet trained children) or midstream sample (toilet trained children)
Urine Microscopy Muddy brown or granular casts is suggestive of acute tubular necrosis Predominant leukocytes is suggestive of acute interstitial nephritis Urinary eosinophils for suspected acute interstitial nephritis Red blood cell casts and/or white blood cell casts are suggestive of acute glomerulonephritis
Proteinuria Total (including glomerular and tubular) protein (protein:creatinine ratio or 24-h urine collection if proteinuria appears significant) Glomerular proteinuria (albumin:creatinine ratio) Tubular proteinuria (ß2-microglobluin, as may be elevated with interstitial nephritis)
Fractional Excretion of Sodium Fractional Excretion of Sodium (FeNa) (%) = (Urine Na/Plasma Na)/(Urine Creatinine/Plasma Creatinine) * 100 *Ensure that units of measurement for urine and plasma Na and for urine and plasma Creatinine are the same* FeNa <1% suggests decreased kidney perfusion with adaptive decrease in GFR/fluid-responsiveness FeNa >2% suggests intrinsic AKI (e.g., ATN)
Fractional Excretion of Urea^{b} Fractional Excretion of Urea (FeUrea)^{b} (%) = (Urine Urea/Plasma Urea)/(Urine Creatinine/Plasma Creatinine) * 100 *Ensure that units of measurement for urine and plasma Na and for urine and plasma Creatinine are the same* FeUrea <35% suggests decreased kidney perfusion with adaptive decrease in GFR/fluid-responsiveness FeUrea >50% suggests intrinsic AKI
Blood Tests
Serum creatinine and document or estimate baseline serum creatinine to calculate AKI severity • Consider correcting serum creatinine for fluid status if there is fluid overload: Corrected Creatinine = Serum Creatinine [1 + (Net Fluid Balance since admission in liters/Total Body Water in liters)]
Further investigations for severity and etiology of AKI Complete blood count If anemia and thrombocytopenia, concern for TMA: send LDH, bilirubin, haptoglobin, blood film (smear). Blood urea nitrogen Sodium, potassium, chloride, bicarbonate, ionized and total calcium, magnesium, phosphate, blood gas (evaluate severity of AKI) Albumin Consider: liver enzymes and INR (if suspect hepatorenal syndrome); auto-immune/vasculitis work-up directed by history and physical exam; drug levels for patients on nephrotoxic medications which can be measured in the blood. If concern of rhabdomyolysis, send serum creatine kinase, urine myoglobin
Tests, if abnormal, suggest acute on chronic kidney disease Iron studies, including ferritin, iron, transferrin, total iron binding capacity, and calculation of percent transferrin saturation (TSAT) Intact PTH 25-[OH]-Vitamin D3
Imaging
Diagnostic Imaging Includes renal ultrasound with Doppler – kidney anatomy, obstruction, vascular thrombosis CT or MR imaging in cases with suspect mass or to rule out stones, as clinically indicated. Other investigations depend on etiology including VCUG, renal MAG3 scan, and DMSA renal scan
Other
Renal Biopsy
AKI Biomarkers (currently commercially available tests for NGAL and for TIMP-2*IGFBP-7 are available)

(in particular proximal tubule) may be more evident. Findings of tubular injury include, but are not limited to: epithelial cell swelling, loss of brush border; cell swelling with vacuolization, characteristic of tubular toxicity, often due to drugs; with more severe injury, flattening of epithelial cells with evidence of mitosis (i.e., cellular regeneration); interstitial edema and/or mild inflammation; different types of casts seen in the tubular lumens.

Importance of Considering Clinical Setting During AKI Evaluation

When evaluating AKI in specific clinical settings, it is important to consider the relevant pathophysiology of AKI within that setting. For example, in the setting of sepsis-induced AKI or cardiac surgery-associated AKI, where ATN may be prominent, oliguria is typical. However, patients treated with cisplatin or ifosfamide for cancer may develop significant direct renal tubular injury, but may not present in the typical manner of other causes of ATN. The primary insult is tubular injury leading to severe abnormalities in tubular electrolyte transport function and urinary concentration. Thus, these patients will primarily present with severe electrolyte disturbances *without* oliguria. Similarly, cardiac patients with a history of prolonged low cardiac output state and kidney hypoperfusion (cardiorenal syndrome) with consistent use of diuretic therapy may have subtle creatinine increase without modifications in urine output. A similar presentation may occur in the setting of aminoglycoside nephrotoxicity. It is important to appreciate these differences, foresee potential complications and not understate the importance of the AKI episode, simply due to lack of oliguria.

Evaluating Serum Creatinine Rise: Distinguishing Adaptive Renal Response from True Tubular Injury

A common diagnostic dilemma occurs when patients present with a rise in SCr associated with evidence of decreased kidney perfusion. Some patients simply require fluid administration to re-perfuse the kidneys, leading to quick return of SCr concentrations to baseline values; these patients likely do not have significant tubular or *structural* injury. Rather, reduction in GFR resulting in the SCr increase is adaptive, to offset the reduction in kidney perfusion. When kidney hypoperfusion is severe, ATN may develop and overly aggressive fluid administration may contribute or lead to fluid overload and respiratory compromise. The ability to differentiate purely functional SCr alterations from structural injury is currently limited, though research in kidney injury biomarkers will likely fill this knowledge gap in coming years, as described in the next section. Until the use of biomarkers of kidney structural damage is widely available, examination of urine microscopy for muddy brown casts may indicate ATN. Calculating the fractional excretion of sodium or urea may also help differentiate between elevations in SCr due to renal hypoperfusion *vs.* ATN (detailed in Table 3) because the inability of tubules to avoid sodium losses or excessive urea losses conceptually implies their structural damage:

Fractional excretion of sodium:
$$(\text{Urine sodium} \times \text{SCr}/\text{Serum sodium} \times \text{Urine creatinine}) \times 100.$$

The fractional excretion of urea is similarly calculated. Sodium and urea fractional excretion should be low ($<1\%$ for sodium and $< 35\%$ for urea) with fluid-responsive elevations in SCr, whereas with ATN, fractional excretion will be higher ($>2\%$ for sodium and $> 35\%$ for urea, Table 3). The fractional excretion of urea may be less affected by diuretic use, whereas fractional excretion of sodium is unreliable in patients receiving diuretics. Blood urea nitrogen (BUN) elevation out of proportion to SCr may also indicate kidney hypoperfusion as an important contributing factor to AKI; however it is important to remember that BUN can be elevated in states of gastrointestinal bleeding and corticosteroid exposure, and does not rule out the presence of ATN.

A recent study proposed a novel way to help differentiate patients with elevation in SCr fulfilling criteria for Stages 1 or 2 AKI, who will *not* progress to a more severe AKI stage from those who will progress [67]. This diagnostic method is referred to as *the Furosemide Stress Test*: a standard dose of 1 to 1.5 mg/kg of furosemide is administered and urine output response is evaluated. In adults, patients with <200 ml of urine output in the first 2 h of the test had the highest risk for severe AKI [67]. Single studies evaluating the furosemide stress test in pediatric populations have shown similar promising results [68, 69].

Many patients have elevated SCr at the time of ICU admission; but not all these patients will go on to develop more severe AKI. The *Renal Angina Index* is a scoring system applied at ICU admission, which was developed and validated to predict risk for severe AKI in ICU children, by combining known AKI risk factors (transplant recipient; mechanical ventilation or vasopressor support) and functional evidence of injury (i.e., SCr rise and fluid overload %; Table 4) [70]. Renal Angina Index derivation and validation studies showed that a score ≥ 8 was highly predictive of the presence of $\geq$ Stage 2 AKI on ICU day 3 [71, 72]. Although the Renal Angina Index has been mainly used for prognostication in the research setting, it highlights the importance of identifying patients at high risk for severe AKI early after ICU admission, which may have significant implications on preventive and anticipatory management.

AKI Injury Biomarkers

In the last 15 years, a large amount of research has been performed to evaluate new diagnostic tests of AKI. As described above, the *functional markers* SCr and urine output do not adequately distinguish presence or absence of actual kidney tissue/structural injury. Moreover, SCr and/or urine output change late in the pathophysiology of AKI, up to 72 h after injury has occurred [73]. These characteristics pose significant challenges to successful interventions to prevent or treat AKI. Serum Cystatin C (CysC) (a non-glycosylated, 13.3-kDa protein belonging to cystatin protease inhibitors, normal value 0.65–0.9 mg/l) is a marker of GFR in a similar way to SCr. Many centers around the world now include CysC measurement as a standard biochemistry test. Cystatin C has many advantages over SCr for evaluation of kidney function, including concentrations do not vary substantially with age, muscle mass, diet, and physical activity [74]; CysC is primarily excreted by glomerular filtration; it undergoes minimal tubular secretion and in the urine it is degraded by proximal tubular

Table 4 Summary of the renal angina index for predicting severe AKI on day 3 of ICU admission[a]

RISK	**Risk Criteria**		**SCORE**
	ICU admission		**1**
	Solid organ or stem cell transplantation		**3**
	Mechanical ventilation or vasoactive support		**5**
INJURY	**ICU admission SCr[b] divided by Baseline SCr**	**% Fluid Overload**	**SCORE[c]**
	Decreased or no change	<5%	**1**
	>1–1.49	5–10%	**2**
	1.5–1.99	10–15%	**4**
	≥2	>15%	**8**
			Renal Angina Index Score ***How to calculate:*** Risk Score (1, 3 or 5) × Injury Score (1, 2, 4, or 8). Score range: 1 to 40 **Renal Angina = Score ≥ 8**

[a] Renal angina index score is calculated 12 h after ICU admission
[b] The highest SCr between ICU admission and 12 h after admission is used
[c] The worst of the SCr/Baseline and %Fluid Overload score is used

cells [74]. These attributes make CysC an ideal and likely more accurate marker of GFR than SCr is [14, 75, 76]. However, the extent to which CysC is more than useful than SCr is to identify *acute changes* in GFR, such as with AKI, is still unclear. Some studies in ICU patients, including those undergoing cardiac surgery, have shown that CysC rises with AKI before SCr does [77–79]. Recently, including acute CysC changes in AKI evaluation has been shown to be a possibly useful adjunct test to predict AKI outcomes in children treated with stem cell transplant [80]. However, CysC remains a *functional marker* (i.e., of GFR), rather than a marker of kidney tissue/structural injury.

Translational research using different animal models of ischemic and nephrotoxic AKI has identified several novel urinary proteins of kidney injury [81–86]. These proteins are either secreted in urine as a result of renal tubular cell injury and extrusion from the cell cytoplasm, or are somehow involved in AKI pathophysiology or cellular repair, allowing for early identification of tissue injury, *before* function is impaired (i.e., before SCr rises or urine output decreases). Table 5 summarizes only some of the biomarkers which have been studied, with reference to their origins and proposed role in AKI [86–100]. AKI biomarkers of tissue injury may be broadly classified into tubular damage biomarkers (mainly filtered urinary proteins normally reabsorbed by uninjured proximal tubular cells, such as beta-2 microglobulin or *N*-acetyl-beta-D-glucosaminidase), biomarkers whose genes are induced or downregulated by AKI (e.g., neutrophil gelatinase-associated lipocalin [NGAL]; interleukin-18 [IL-18]; kidney injury molecule-1 [KIM-1]), or biomarkers of cell cycle arrest (e.g., tissue-inhibitor of metalloproteinases-2 [TIMP-2]; insulin-like growth factor binding protein-7 [IGFBP-7]). This chapter does not discuss an

Table 5 Some recently studied biomarkers of AKI which are upregulated in response to injury (This biomarker list is not exhaustive, but merely represents some recent or commonly published biomarkers in pediatrics. Many more AKI biomarkers have been discovered and undergone initial human validation study)

Biomarker	Kidney injury location	Mechanism of action upon injury
Neutrophil gelatinase-associated lipocalin (NGAL) **commercially available assay*	Proximal tubules, likely distal	Upregulated, released in urine/plasma. Action unknown, appears protective: chelates iron from damaged tubules preventing free radical formation, less cell death
Interleukin-18 (IL-18)	Proximal tubules	Produced by proximal tubule cells: upon injury, caspase-1 cleaves pro-Il-18 inactive form to active form which exhibits pro-inflammatory properties
Kidney injury molecule-1 (KIM-1)	Proximal tubules	Promotes apoptotic and necrotic cell clearance: upregulated and shed into the urine and extracellular space
L-type fatty acid binding protein (L-FABP)	Proximal tubules	Upregulated, binds lipid hydroperoxides and other reactive oxygen species
Hepcidin-25 (Hep-25)	Thick ascending limb, collecting ducts. Cellular: apical cell pole.	Inhibits intestinal iron absorption and release from macrophages/hepatocytes; upregulated by inflammation
Product of tissue inhibitor of metalloproteinases-2 *and* Insulin-like growth factor binding protein-7 (TIMP-2 X IGFBP-7) **commercially available assay*	Two G1 cell cycle arrest biomarkers: TIMP-2 is a metalloproteinase inhibitor; IGFBP7 is an insulin-like growth factors binding protein	Expressed in renal tubular cells with injury, leading to blocking the effects of cyclin-dependent protein kinase complexes on cell cycle promotion, G1 cell cycle arrest

exhaustive list of AKI biomarkers being studied.

Tubular damage biomarkers have demonstrated modest success at best for AKI diagnosis prior to SCr rise. Biomarkers induced or downregulated with renal tubular injury have variably shown the highest promise for early AKI diagnosis. Pediatric AKI biomarker research to date has focused mostly (but not only) on urine NGAL, IL-18, KIM-1, and liver-type fatty acid binding protein (L-FABP) [99]. Several studies in children undergoing cardiac surgery, admitted to the critical care unit, treated with intravenous contrast or other clinical AKI scenarios (including patients undergoing stem cell transplant, treated with chemotherapies or other nephrotoxins), demonstrate these urine biomarker concentrations increase up to 48 h prior to SCr in patients with AKI and/or predict poorer outcomes and morbidity [80, 86, 93, 95–97, 101–104]. Furthermore, increasing elevations in these biomarkers may predict development of worse AKI severity [80, 91, 103]. Until recently, one of the challenges in moving past the research phase of novel AKI biomarkers has been the lack of rapid testing methods or of biomarker measurement methods available for use on standard biochemistry platforms. NGAL is now available for measurement using standard lab platforms and several centers across the world have begun utilizing urine NGAL measurement in clinical care. This is fortunate, as urine NGAL is the AKI biomarker which has the strongest evidence for early detection of AKI in children, especially in children undergoing cardiac surgery. Although more research is needed to identify optimal thresholds for AKI diagnosis in children with AKI from different causes, proposed urinary NGAL diagnostic concentration thresholds for mild AKI and for severe AKI are $\geq$50 ng/ml and $\geq$ 150 ng/ml, respectively [80, 105, 106]. The multiplicative product of urinary concentrations of TIMP-2 and IGFBP-7 (TIMP-2*IGFBP-7) has been evaluated for early AKI diagnosis in critically ill adults [99, 100]; a commercially available rapid testing assay (the "Nephrocheck®") is now available and approved in the USA for predicting AKI in critically ill adults at ICU admission. Several studies have recently been performed in children undergoing cardiac surgery and other patient populations, showing promise for clinical application in children [100, 104, 107, 108]. Hopefully, ongoing validation studies and studies determining the best diagnostic thresholds in children will be expedited by the fact that a commercially available assay is available. Although current AKI biomarker utility is still mainly used for research, future AKI definitions will likely incorporate markers of kidney tissue injury in addition to markers of kidney function (i.e., SCr and UO) [109, 110]. This will result in a huge change in how AKI is approached, providing the ability to differentiate fluid-responsive AKI or quickly reversible AKI or benign forms or renal dysfunction from true acute kidney tissue injury and the ability to prognosticate progression of AKI to more severe forms.

An important concept in moving toward clinical application of novel AKI biomarkers is that these diagnostic tests are most likely to be useful when applied to patients who are *at substantial risk* for AKI, versus widespread non-selective application; that is, testing the right patient at the right time. One major advancement in this field has been combing application of AKI risk scores, like the Renal Angina Index (described above), with novel AKI biomarkers. When AKI biomarkers (particularly, NGAL) were measured in children with ICU admission Renal Angina Index score $\geq$ 8, prediction of Stage 2 or worse AKI by Day 3 of ICU admission drastically increased, being near perfect [101]. In another example, when the clinical risk assessment provided by the Furosemide Stress Test was combined with urine AKI biomarkers, prediction of severe AKI development was significantly improved [111]. Once new AKI biomarkers become approved for bedside clinical testing, they may also be useful to show the effectiveness or toxicity of novel AKI therapies.

Management

Prevention

Ideally, measures to prevent AKI occurrence by avoiding risk factors in susceptible patients should be instituted before AKI develops (primary prevention). However, unfortunately, the pediatric nephrologist often becomes involved in patients with AKI well after the injury has occurred. A recent study in children treated with aminoglycosides for a cystic fibrosis pulmonary exacerbation, demonstrated that increasing SCr monitoring during therapy (daily, versus at the medical team's discretion), was associated with an increase in detection and earlier identification of AKI [35]. This makes sense; if kidney function is monitored more closely in at-risk patients, the likelihood of detecting AKI will increase. Potential benefits include earlier diagnosis, more timely avoidance of inappropriate fluid administration and other AKI risk factors (e.g., other nephrotoxins), and possibly reduction of ultimate AKI severity. Thus, the nephrologist may work with other medical services to identify specific patients at risk for AKI and suggest renal function monitoring strategies with the goal of early identification and further kidney injury avoidance. Another study demonstrated that in non-critically ill hospitalized children, the presence of three or more nephrotoxins increased the risk of developing AKI [33]. Based upon this work, one study implemented an electronic health record (EHR)-guided program which included daily SCr monitoring program and pharmacy-guided recommendations for all non-critically ill patients receiving three or more nephrotoxic medications or an intravenous aminoglycoside for more than 3 days [36]. This program, discussed in detail in chapter "Nephrotoxic Medications," is called the "Nephrotoxic Injury Negated by Just-in time Action (NINJA)" program; at the initial center where NINJA was developed, adherence to appropriate and recommended SCr monitoring was greater than 99%, and they observed a 42% reduction in AKI days. In a recently published nine-center NINJA application and validation study, an overall 24% reduction in nephrotoxic-associated AKI rate was observed [112]. In centers where such a systematic, EHR-guided program may not be feasible, the nephrologist may still significantly impact AKI avoidance by consulting with other medical specialty colleagues to provide education regarding nephrotoxin avoidance and kidney function monitoring or minimization.

Other attempts have been made to leverage EHRs to predict imminent AKI or to alert regarding its early detection [113, 114]. Electronic "sniffers" or "clinical decision supporting systems" have been evaluated as tools to achieve primary and secondary AKI prevention. Additionally, EHR-based models may contribute to the stratification of patients in whom further diagnostic measures could be performed (e.g., AKI biomarkers), enhancing the synergy between two predictive modalities and improving the respective predictive accuracy [115]. Studies including thousands of hospitalized patients were able to show the efficacy of these systems in reducing severe AKI incidence by simply increasing clinicians' awareness regarding the presence or the worsening of kidney function [116, 117]. The role of these systems in critically ill children is currently unknown but a wise application of organ failure alerting will possibly be a common application in electronic charts in the years to come.

Measures to prevent *further* kidney injury once AKI is present (secondary prevention) are instrumental in AKI management, namely, by avoidance of [1] hypotension (including provision of appropriate inotropic support) and [2] nephrotoxic medication use, with re-evaluation of the need for nephrotoxins and closely following serum concentrations when possible. A recent consensus publication from the 22nd Acute Dialysis Initiative conference [3], which was focused on quality improvement in AKI, nicely outlined several potential guidelines and recommendations for primary and secondary prevention of AKI, summarized briefly in Fig. 1. This document highlights the importance of (1) primary prevention (Fig. 1a): assessing *all* hospitalized patients for *risk* of developing AKI (determining whether their clinical history, pre-admission medications, acute illness, or other factors place them at risk for AKI) and if they are at risk, ensuring that appropriate measures are taken to *diagnose* AKI (e.g., SCr monitoring, urine output measurement); and (2) secondary prevention (Fig. 1b): Once AKI is diagnosed, to ensure that the severity of AKI is appropriately evaluated and that measures are actively taken to diagnose the cause, minimize further injury and promote recovery.

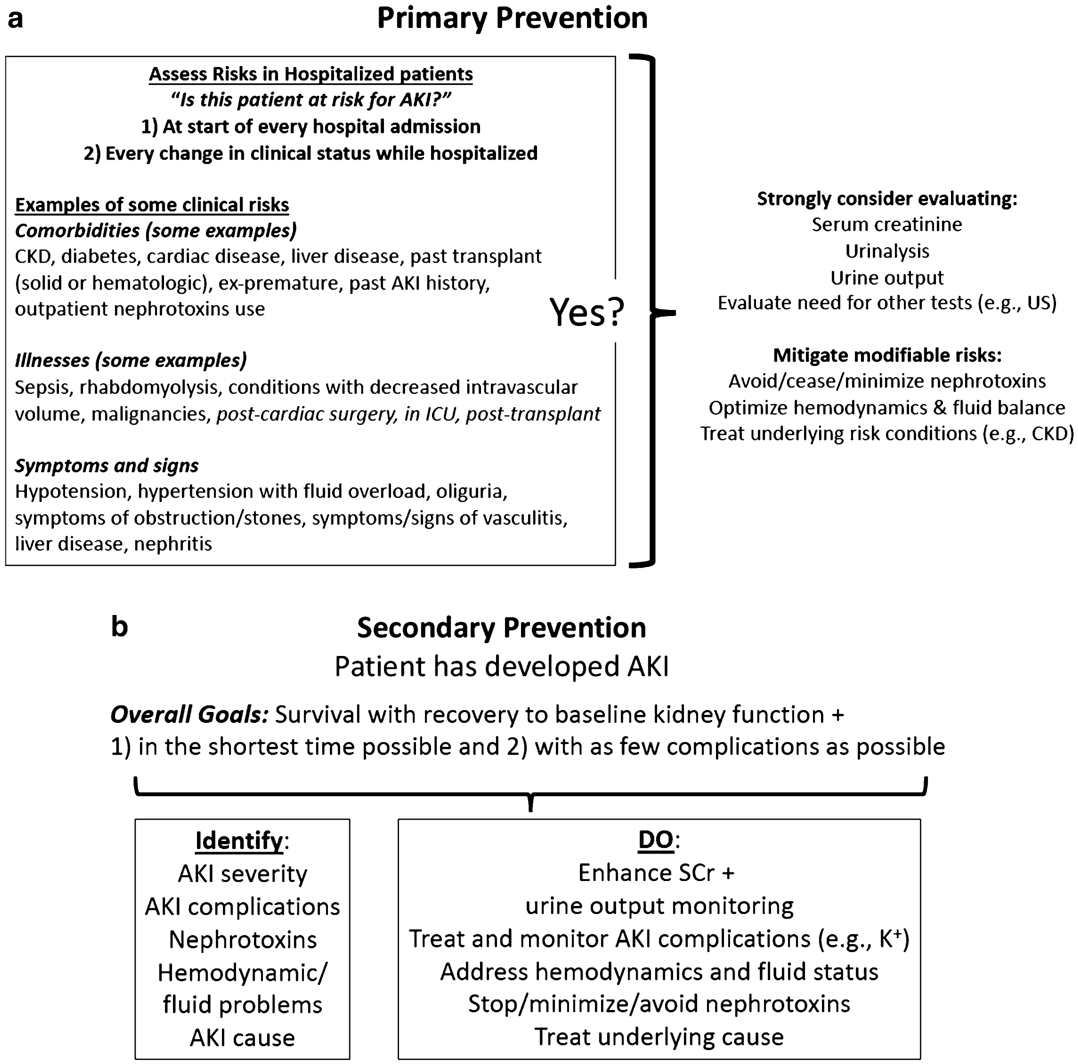
a
Primary Prevention
Assess Risks in Hospitalized patients
"Is this patient at risk for AKI?"
1) At start of every hospital admission
2) Every change in clinical status while hospitalized
Examples of some clinical risks
Comorbidities (some examples)
CKD, diabetes, cardiac disease, liver disease, past transplant (solid or hematologic), ex-premature, past AKI history, outpatient nephrotoxins use
Illnesses (some examples)
Sepsis, rhabdomyolysis, conditions with decreased intravascular volume, malignancies, post-cardiac surgery, in ICU, post-transplant
Symptoms and signs
Hypotension, hypertension with fluid overload, oliguria, symptoms of obstruction/stones, symptoms/signs of vasculitis, liver disease, nephritis
Yes?
Strongly consider evaluating:
Serum creatinine
Urinalysis
Urine output
Evaluate need for other tests (e.g., US)
Mitigate modifiable risks:
Avoid/cease/minimize nephrotoxins
Optimize hemodynamics & fluid balance
Treat underlying risk conditions (e.g., CKD)
b
Secondary Prevention
Patient has developed AKI
Overall Goals: Survival with recovery to baseline kidney function +
1) in the shortest time possible and 2) with as few complications as possible
Identify:
AKI severity
AKI complications
Nephrotoxins
Hemodynamic/ fluid problems
AKI cause
DO:
Enhance SCr + urine output monitoring
Treat and monitor AKI complications (e.g., K+)
Address hemodynamics and fluid status
Stop/minimize/avoid nephrotoxins
Treat underlying cause

Fig. 1 (**a**) Proposed approach to primary and secondary hospital-AKI prevention, Modified from the Acute Dialysis Quality Initiative Consensus Statement. (**b**) This simplified approach was modified from the Acute Dialysis Quality Initiative 22nd conference consensus publication on quality improvement targets in AKI. A pediatric-specific document will be developed in coming years

Fluid Management

Careful attention to fluid management is critical to mitigate AKI complications. Patients with decreased kidney perfusion and adaptive decrease in GFR as the cause for SCr rise will often respond to fluid resuscitation, whereas patients with ATN should be treated with volume restriction, to prevent development of worsening fluid overload, or may require diuretics to mitigate fluid overload. Thus, fluid prescription for children with AKI is directed by the individual clinical situation. Generally, a safe starting point, assuming fluid resuscitation has been performed, is to provide insensible losses (400 ml/m^2 of body surface area in patients with normal basal metabolic rate, higher in febrile patients and lower for ventilated patients who have lower respiratory losses), plus replacement of ongoing losses (urinary, gastrointestinal, chest tubes). This fluid prescription will generally be appropriate to *maintain* current volume status in patients who require fluid restriction and in those with polyuria, but will not be appropriate in the setting of acute volume depletion. To promote the achievement of negative balance in patients exhibiting fluid overload, less than the total urine output may be replaced. After a period of observation, a fixed daily fluid prescription may be prescribed, while ensuring frequent re-evaluation. Because of the important potential deleterious effects of fluid overload on outcomes described above, it is vital to always remember to think of overly aggressive fluid provision in patients with AKI as potentially detrimental. Judicious administration of fluids coupled with very frequent re-evaluation of volume status allowing for timely initiation of fluid removal when needed may lead to avoidance of these negative fluid overload effects.

Prospective randomized studies of adult patients at risk for ATN have shown that intravenous furosemide is unlikely to reverse oliguria and large retrospective studies in adults have not demonstrated improvements in outcomes in patients with AKI [118, 119]. The KDIGO AKI guideline recommends using diuretics to manage fluid overload and not to prevent or treat AKI [1]. However, maintaining urine output clearly facilitates the delivery of appropriate nutrition, without further worsening fluid overload. Typically, loop diuretics are used to maintain urine output (most commonly furosemide, but bumetanide is often used as well) either as an intermittent bolus or as a continuous infusion. Especially if there is evidence of benefit to maintaining urine output, adding a thiazide diuretic or metolazone, which targets the distal segment of the renal tubules, may be helpful. Use of diuretics to maintain urine output needs to be balanced with careful and frequent evaluation of volume status and frequent evaluation for success or non-success to avoid delay in KRT initiation. In other words, if diuretics are being used to maintain urine output in order to provide nutrition and avoid fluid overload, then these two desired outcomes should be measured and evaluated frequently. Another situation where volume control is crucial (i.e., critical need to avoid volume overload) is when “congestive” kidney dysfunction is present (e.g., patients with right ventricular dysfunction, severe congestive heart failure, or some children after complex cardiopulmonary bypass surgery). In these patients, elevated central venous pressure can significantly contribute to reduced GFR and likely to kidney injury, which strongly suggests that aggressive and timely management of volume overload (i.e., diuresis and/or KRT) may contribute to prevention and/or recovery from AKI [120, 121]. In such patients, close communication between the nephrologists and intensivist is crucial.

Electrolyte Management

Electrolyte management in AKI includes both acute and subacute management and is also dependent on the cause of AKI. Patients with oligo-anuric kidney failure or severe ATN should not receive potassium or phosphorus unless they exhibit hypokalemia or hypophosphatemia. Sodium intake should be restricted to 2–3 mEq/kg body weight per day, together with fluid restriction, to prevent sodium and fluid retention with resultant hypertension. Conversely, patients with AKI secondary to nephrotoxic medications or interstitial nephritis may demonstrate polyuria, placing them at risk for fluid and electrolyte losses. Polyuria in this setting may lead to salt or other electrolytes wasting, and may require higher administration than normal. In order to safely achieve these goals, it is ideal to request insertion of a urinary catheter and obtain weights and serum electrolyte levels at least daily.

Acute non-dialytic management of electrolyte problems due to AKI includes management of severe acute hyperkalemia which can lead to deadly cardiac arrhythmias. Intravenous calcium may be used to mitigate these effects, and measures to increase potassium entry into the cells, such as sodium bicarbonate (if the patient is acidotic), beta-2 agonists or insulin with dextrose, can be given. Severe hypocalcemia may also cause cardiac conduction defects and should be corrected judiciously, especially in the setting of severe hyperphosphatemia, to avoid crystal formation. Moreover, the administration of sodium bicarbonate for correction of hyperkalemia may exacerbate hypocalcemia. Hyperphosphatemia, if not severe and not associated with severe hypocalcemia, may be managed with phosphate binders and restriction.

Pharmacological Therapy

There is no proven specific pharmacologic treatment for AKI. However, preservation of renal perfusion with appropriate inotropic agents is essential in critically ill patients unresponsive to volume repletion [122]. The effects of dopamine, a commonly used inotropic agent in children, are varied and complex, leading to controversy with respect to its utility in the setting of AKI. Dopamine increases renal plasma flow and sodium excretion at low or so-called renal doses of 0.5 to 2 mcg/kg/min. However, these effects are short-lived [123]. At increasing doses, dopamine binds beta- and then alpha-adrenergic receptors, rendering it difficult to ascertain whether any observed renal benefit from dopamine occurs as a result of its dopaminergic or inotropic effect. Well-designed prospective randomized studies of adult patients at risk for ATN have seriously called into question the utility of "renal-dose" dopamine in reversing oliguria [124], and most centers have abandoned its use for this purpose.

The benefits of dobutamine and norepinephrine for patients with AKI reside in their ability to increase cardiac output, leading to an increase in renal blood flow [125]. Vasopressin increases systemic vascular resistance by direct action on the vascular smooth muscle cells. Vasopressin has been shown to be especially effective in maintaining renal perfusion in patients with septic shock who were unresponsive to catecholamine therapy [126].

Several recent systematic and narrative reviews have summarized many different therapeutic targets and drugs that have been studied, for which trials are ongoing or which are planned for human trials [127, 128]. Potential therapeutic targets for AKI include, but are not limited to, antioxidants, anti-apoptotics, anti-inflammatories, increasing renal blood flow and oxygen delivery, renal tissue repair (i.e., stem cells), and more conservative therapies such as hyperglycemia avoidance. Only a few are described herein. Some pharmacologic agents, such as calcium channel-blockers and *N*-acetyl cysteine, have been studied quite extensively in adults but have shown no substantial benefit on mortality or kidney outcomes [129, 130]. The selective dopamine A-1 antagonist fenoldopam may improve urine output in children undergoing cardiopulmonary bypass surgery [131]. Some studies suggest that nesiritide (a human natriuretic peptide) may have favorable renal hemodynamic effects and increase urine output after cardiac surgery [132]. However, clinical trials are lacking in children for most of these medications. Several studies in neonates with perinatal asphyxia have suggested that a single dose of theophyilline (an adenosine receptor antagonist) early after birth resulted in improved renal function and urine output [133]. There is also emerging, but tenuous and conflicting, evidence that adenosine receptor antagonists may prevent or reduce severity of AKI in children undergoing cardiac surgery [134, 135]. A recent meta-analysis on the use of pharmacological therapies for AKI in children undergoing cardiac surgery showed that the use of intravenous acetaminophen and the use of dexmedetomidine (an alpha-2 adrenergic receptor antagonist which many enhance intrarenal hemodynamics, prevent tachyarrhythmias and have preconditioning effects) may be beneficial to reduce postoperative AKI incidence [127, 136]. However, much more research is needed. Hopefully, with improved diagnostic assessment and increased ability to identify patients at highest risk for AKI (and thus, most likely to benefit from therapies), future research will identify pharmacological therapies which improve kidney outcomes and mortality in children with AKI.

Kidney Replacement Therapy

A substantial amount of literature has demonstrated that acute KRT for AKI may be provided safely to children of all ages. The biggest decisions reside in what modality of KRT to use and

Table 6 Advantages and disadvantages of different acute kidney replacement therapy modalities

Modality	Advantages	Disadvantages
Intermittent hemodialysis	Short treatment times Accurate ultrafiltration	Vascular access necessary Hemodynamic instability Heparin anticoagulation
Peritoneal dialysis	No need for vascular access Minimal equipment needs Minimal training needs Feasible in small infants Continuous treatment	Less efficient than hemodialysis or continuous renal replacement therapy Variable ultrafiltration dependent on blood pressure
Continuous renal replacement therapy	Accurate ultrafiltration that can be altered to account for changes in intake/patient blood pressure Smaller circuit volumes Citrate anticoagulation	Vascular access necessary

when to initiate it. No studies comparing dialysis modality and dose have been performed in children. It is therefore important to have an understanding of the use of all KRT modalities available in children. This section will focus mainly on technical aspects of continuous kidney replacement therapy (CKRT), since intermittent hemodialysis (IHD) and peritoneal dialysis (PD)) technical aspects are covered in chapters related to maintenance dialysis provision for children with end-stage kidney disease.

When fluid or electrolyte abnormalities are severe, KRT becomes the main treatment of choice for AKI. Traditional indications for KRT in AKI are hyperkalemia, severe hyperphosphatemia (especially if accompanied by hypocalcemia), severe metabolic acidosis or fluid overload, and symptoms of uremia. Non-kidney indications include prevention or treatment of tumor lysis syndrome to avoid uric acid or calcium-phosphate nephropathies, removal of toxins (ingestions or inborn errors of metabolism). While little has changed in terms of acute indications, the goal of prevention or early treatment of fluid overload using KRT has become more prevalent [38]. Acute KRT can be provided as IHD, PD, or CKRT. There are advantages and disadvantages to each of these modalities.

Modality Choice

Several factors, including dialysis indication and factors related to the patient's clinical status, determine the optimal KRT modality [137]. In general, factors determining optimal modality include patient size, hemodynamic stability, and institutional expertise. Table 6 outlines various advantages and disadvantages of the different acute KRT modalities. PD and CKRT are better suited for patients with hemodynamic instability, since daily total ultrafiltration goals can be achieved over a 24-h period instead of a 3–4 h IHD treatment. In patients with disrupted or severely scarred peritoneal membrane, PD may not be possible. Acute drug intoxications and hyperammonemia secondary to inborn errors of metabolism are most reliably treated with IHD since rapid drug removal is important to prevent morbidity and IHD is the most efficient KRT modality. However, several studies have shown that CKRT performed using very high solute clearance rates can also be effective for acute removal of ammonia and some drugs [138]. CKRT can be very effective for removal of some protein-bound drugs if albumin is added to dialysis fluid [139]. Sustained low-efficiency daily dialysis (SLEDD, often referred to as SLED for sustained low-efficiency dialysis) refers to using HD machines to administer HD over longer periods (e.g., 6–12 h), and is used in many centers with similar indications to the use of CKRT (e.g., hemodynamically unstable patients) [140]. Although duration and prescription of SLEDD may vary by patient needs and center, in general, dialysis fluid flow rates (and in older children, sometimes blood flow rate) are slower than in standard IHD (e.g., dialysis fluid rate of 100 ml/min instead of

500 ml/min). Recently, a somewhat interchangeable nomenclature for SLED was proposed: prolonged intermittent renal replacement therapy (PIRRT) [141]. This term may better reflect the flexibility of this modality, whereby blood flow, dialysis fluid rate, and duration and frequency of therapy can be modified according to the patient needs. Finally, the particular center's expertise and nursing support availability should play a major role in determining the optimal KRT modality.

Intermittent Hemodialysis

IHD is only discussed briefly here, as most details on materials, procedures, and prescription are discussed in a dedicated chapter. Vascular access issues are similar to what is discussed below in the section on CKRT. Acute IHD can be performed using similar machinery and dialysis solutions as in chronic HD, which means the need for a portable reverse osmosis device, purified water, and nurses specialized in performing HD. In hemodynamically stable patients with primary renal disease (e.g., acute glomerulonephritis, hemolytic-uremic syndrome), or in patients with inborn errors of metabolism or intoxications, IHD may be preferred because very efficient small solute clearance can be obtained within a short period and these patients will generally tolerate the procedure well. In patients with severe uremia, who are being treated with IHD for the first time, a relatively low clearance (aiming at a urea reduction ratio ≈ 30%) should be prescribed, in order to avoid the dialysis disequilibrium syndrome. If blood urea nitrogen is very high, intravenous mannitol or sodium profiling during dialysis may be helpful to avoid this complication. Heparin anticoagulation is generally used in IHD, which may complicate the clinical picture for patients with a coagulopathy. Heparin-free IHD can be achieved by performing frequent (approximately every 30 min) flushes with normal saline to the hemodialysis circuit. Hemodynamically unstable patients will often not tolerate a session of IHD, and, importantly, very tight fluid control will be necessary in between dialysis sessions, which may impair ability to provide adequate amounts of nutrition. In these patients, continuous modalities are preferable.

Peritoneal Dialysis

Acute peritoneal dialysis is a continuous dialytic therapy which requires much less technical expertise, expense, and equipment compared to IHD and CKRT. PD catheters can be placed quickly and easily. In centers with expertise, a non-tunneled PD catheter can be placed percutaneously at the bedside [142]), but these may have a high tendency to leak when inserted in centers with less experience in this procedure. Placing catheters surgically is preferable, similar to chronic PD catheter placement, and may avoid problems with peritoneal leak and resultant risk of infection [143]. Perioperative prophylactic intravenous antibiotics (e.g., cefazolin 15–20 mg/kg) are advisable. Flushing the PD catheter with dialysis solution and heparin after PD catheter insertion, until the effluent is clear, may reduce the risk of catheter blockage and malfunction and also allows for an early evaluation of catheter function. Several centers practice partial omentectomy when inserting acute PD catheters surgically, in order to minimize post-insertion catheter malfunction.

A recent trend toward providing PD therapy earlier in the post-cardiopulmonary bypass has been reported [144]. Improved survival with early PD initiation may result from prevention of fluid overload and potentially from abdominal decompression. Some authors suggest that improved survival with early post-cardiac surgery PD initiation may result from increased clearance of CPB-induced pro-inflammatory cytokines [145]), although further study is required to support this hypothesis. A study in infants undergoing cardiac surgery comparing patients who had intraoperative PD catheter placement (i.e., "prophylactic" PD) versus infants who did not showed that achievement of negative fluid balance and of extubation occurred more quickly in the intervention group [146]. A single center randomized controlled trial showed superior fluid removal with PD compared to standard dose diuretics [147]. The benefits of prophylactic PD catheter insertion on postoperative outcomes remains controversial [148, 149], but several centers perform this practice in high risk surgeries to aid in postoperative fluid management. Of note, for patients with some primary kidney diseases (e.g., glomerular diseases, hemolytic uremic syndrome) that do not require a critical care setting but who may be expected to require KRT for at least several days, PD may be preferred and should always be considered, because it is well tolerated and allows vessel preservation.

Standard PD equipment (including cyclers, or manual setups and solutions) can be used to perform dwells. Initial dwell volumes should be limited to 10 ml/kg of patient body weight in order to minimize intra-abdominal pressure, especially in patients with severe pulmonary dysfunction, and potential for fluid leakage along the catheter tunnel. Heparin is typically instilled in the dialysis fluid to prevent fibrin formation, for at least 48–72 h. Intra-dialysis fluid heparin should be continued for as long as fibrin is present in the dialysis effluent. Some centers also instill cefazolin in the dialysis solution for 48–72 h to help prevent infection. In patients receiving antibiotics, the use of antifungals should be considered, as is the practice for patients receiving chronic PD. If the patient's clinical status is stable, allowing 1–2 days for healing, prior to PD catheter use, may be helpful to prevent leakage; however, this has never been formally studied. In most patients in the acute setting, PD needs to be initiated very quickly.

Physiologic dialysis solutions should be used, and typically, a lower dextrose concentration solution is used initially (e.g., 1.36% Physioneal solution or 1.5% Dianeal solution); the resulting ultrafiltration can be evaluated after a few dwelling cycles and eventually, if required, the dextrose concentration can be increased. Fill volumes for dwells can be increased progressively, but slowly after several days, if there is no PD catheter site leak and if the PD catheter insertion was uncomplicated (particularly for tunneled catheters). In the acute setting, hourly cycles (with approximately 45 min of total dwell time) may be used and PD may be performed for 24 h/day. The dextrose concentration of the dialysis fluid and/or the cycle duration (as low as 15–20 min dwell time if needed) can also be manipulated to achieve the desired solute and/or fluid removal. PD delivers less efficient solute removal than IHD or CKRT and fluid removal (ultrafiltration) can be unpredictable; however, its relative simplicity allows for KRT provision in settings lacking pediatric dialysis specific support and personnel. Complications of acute PD include peritonitis, leakage around the tunnel or poor catheter function (i.e., blockage or inability to drain due to several reasons, similar to chronic PD complications). Moreover, especially relevant to critically ill patients, hyperglycemia may occur due to dextrose-containing dialysis solutions, and protein losses will occur, mandating increased protein intake to avoid protein-malnutrition. Hypothermia may also be a complication if dialysis solutions are not warmed.

Although not discussed in detail here, it is worth mentioning the possibility of performing what has been referred to as continuous flow peritoneal dialysis (or CFPD)[150, 151]. This therapy requires insertion of two PD catheters (although the use of one PD catheter and one smaller peritoneal drainage tube has been described); alternatively, the use of a double lumen PD catheter has also been described. The abdomen is initially filled with a predetermined volume of dialysis solution (e.g., 10 ml/kg) via the "inflow" PD catheter, after which "continuous flow" of dialysis solution (into the abdomen via the inflow catheter and out of the abdomen via the outflow catheter) at relatively high flow rates. Different flow rates are described in the referenced articles, but can range, for example, from 40 ml/h to several hundred or 1000 ml/h, depending on whether high solute clearance is needed versus steady, continuous clearance. The "outflow" rate can be increased, relative to the inflow fluid rate, to achieve the desired net patient ultrafiltration. Different methods for achieving the desired dialysis fluid inflow and outflow rates have been described, including using CKRT machines; intravenous pump machines; using gravity for the fluid outflow collection bag (which requires very careful monitoring). In addition, it is essential to regularly empty the abdomen, as fluid absorption via the peritoneal membrane is not predictable (e.g., the patient may have 200 ml/h flowing continuously through the abdomen, which is otherwise empty due to absorption). Moreover, PD catheter obstruction can be detrimental to the patient and must be monitored very closely. Severe electrolyte imbalances may occur, thus these patients should be monitored as closely as monitoring is performed for patients receiving CKRT. The main advantages of CFPD are that both solute clearance and ultrafiltration rates achievable are much higher than what is feasible with traditional, single-catheter PD. This therapy thus offers an alternative to CKRT when CKRT equipment is unavailable. Another advantage is for very small infants, in whom both CKRT and larger peritoneal fluid volumes may not be tolerated or feasible.

Continuous Kidney Replacement Therapy

Various CKRT aspects, including dose, dialysis/replacement fluid composition, and anti-coagulation methods, have been studied in recent years. The term CKRT generally refers to one of three treatment modalities: continuous venovenous hemodialysis (CVVHD), continuous venovenous hemofiltration (CVVH), or continuous venovenous hemodiafiltration (CVVHDF). In each of these CKRT modalities, dialysis or replacement fluid is generally not from a water source, like in IHD; rather, solution bags are hung on the CKRT machine and delivered to achieve solute clearance. In CVVHD, like in IHD, a physiologic dialysis solution is run countercurrent to blood flow through the hemofilter. The mode of solute removal is by diffusion and small solutes are removed effectively. The CVVH modality uses a *replacement* solution, which is similar in composition to dialysis fluid, but is infused into the CKRT circuit (and thus into the bloodstream of the patient) either pre-hemofilter (pre-dilution) or post-hemofilter (post-dilution). The same volume of fluid is removed by ultrafiltration simultaneously through the hemofilter in order to achieve solute removal by convection (solute drag). Small solute removal efficiency with CVVH by convection is similar to small solute clearance with CVVHD by diffusion, but larger molecules with molecular weights greater than 1000–10,000 Daltons are removed more efficiently with CVVH. In CVVHDF, both dialysis and replacement solutions are used to achieve solute removal. In all forms of CKRT, additional fluid volumes to the dialysis and/or replacement volumes are removed by ultrafiltration, with the goal of controlling fluid balance according to patients' needs.

Vascular Access

A well-functioning vascular access is essential to the success of IHD or CKRT. The most common sites for acute catheter placement are the internal jugular, subclavian, and femoral veins. Avoiding the subclavian vein is preferable to prevent subclavian vein stenosis in patients who may need permanent vascular access in the ipsilateral upper extremity. The femoral vein provides the advantage of relatively easy and prompt access. However, with the availability of skilled surgeons or intensivists and ultrasound guidance, internal jugular venous placement is also attainable safely. Patient characteristics may also influence the choice of catheter placement. For example, in patients with severe ascites or with a suspected need for a future kidney transplant, internal jugular placement may be preferable, whereas in patients receiving high-frequency oscillatory ventilation, femoral placement will be technically easier and reduce the risk of pneumothorax associated with internal jugular venous access.

A study of adult patients reported increased recirculation and worse performance in catheters placed in the femoral vein versus subclavian or jugular veins [152], and pediatric data suggest that internal jugular access is associated with longer CKRT circuit survival than femoral or subclavian access, independent of anticoagulation method [153]. Catheter size should also be matched to patient size [154]. However, smaller catheters (five French double lumen catheters) are associated with very poor circuit survival, due to clotting and poor blood flow and should be avoided if possible. Of note, with the recent availability of CKRT machines designed for very small infants, the need for traditionally high blood flows may not be needed and thus, vascular access should also be adapted to the technology being used. In these novel CKRT machines developed for use in infants ("miniaturized circuits," described below) use of much smaller

Table 7 Examples of vascular access catheter sizes by size of the patient for performing acute CKRT. (This list is simply an example of catheter selection (without commercial product names), specifically designated by patient size. It is important for each center to be aware of the different vascular access catheters available to them)

	Weight	Catheter
Internal jugular (neck) catheters	<5 kg	8Fr (sometimes 6.5Fr)
	5 kg to <20 kg	10Fr
	20 kg to <35 kg	12Fr
	>35 kg	13.5 or 15.5 Fr
Femoral catheters	<5 kg	6.5Fr (10 cm)
	5 to <20 kg	8Fr (12.5 cm) 10Fr (12 cm) if close to 20 kg
	20 to <40 kg	10Fr (15 cm)
	>40 kg	11.5Fr (13.5 cm or 16 cm)

catheters (including five French double lumen catheters) is feasible [155]. Maintaining access patency between CKRT sessions using heparin or citrate catheter locks is essential to prevent access clotting. Table 7 provides some examples of catheter choice by patient size; however, different centers will have different catheter choices available.

Machines and Hemofilters

Modern CKRT machines are generally user-friendly and consist of computer modules from which physicians may choose the CKRT modality. Accurate ultrafiltration with volumetric/gravimetric control incorporated into machines and disposable lines, and circuits and dialyzers, sized for the pediatric weight spectrum have made CKRT safer in children. Accurate UF and blood flow rates are crucial for pediatric KRT since the extracorporeal circuit volume can comprise more than 15% of a small pediatric patients' total blood volume and small UF inaccuracies may represent a large percentage of a small pediatric patient's total body water. Most of these machines also contain blood air leak detectors, a blood pump, dialysis fluid, and replacement fluid pumps. CKRT machines may also be connected to other extracorporeal machines simultaneously, including ECMO circuits and plasmapheresis devices. This aspect of CKRT will not be discussed herein but is described in the literature [156, 157].

Hemofilters are available in different sizes, as measured by internal membrane surface area, from as low as approximately 0.2 m^2 to over 1 m^2, and range from 60 to over 150 ml of blood volume. Some machines have specific hemofilters associated with them, whereas others are more versatile and can accommodate different hemofilters. Moreover, not all hemofilters are available in all countries. It is thus important to be aware of what hemofilters are available and to have as wide a size selection as possible to fit the range of weights (<3 kg to 100 kg) of patients being treated. Thus, hemofilters are chosen based on the type of machine being used and the size of the patient. Despite the smaller sizes of some hemofilters, delivery of CKRT to infants or small patients entails a significant portion of their blood volume to be pumped through the extracorporeal circuit. Therefore, extracorporeal circuit volumes that comprise more than 10–15% of patient blood volume (or even less, if the patient is hemodynamically unstable) should be primed with blood to prevent hypotension and anemia. When the extracorporeal circuit volume is close to 10% of the patient's blood volume, if the patient is severely anemic or is hemodynamically unstable, priming the circuit with blood or with 5% albumin may also be needed. CKRT hemofilters are made of polysulfone or of acrylonitrile (such as the AN69). AN69 membranes are associated with risk for bradykinin release syndrome (which may cause a range of mild to profound hypotension). Bradykinin release syndrome appears to be worse when acidic blood (packed red blood cells in the blood prime) comes into contact with the AN69 hemofilter. Several methods are described to mitigate this reaction, including administration of bicarbonate together with the blood prime, administration of intravenous calcium gluconate when initiating CKRT (to prevent hypocalcemia) as well as performing "pre-dialysis" for 5–10 min of the blood prime solution, before connecting the patient to the CKRT machine [158]. In the latter method, the blood is circulated through the machine against dialysis fluid in order to normalize pH, calcium, and potassium of the blood. In some countries, protein-coated AN69 membranes are available, which greatly reduces the incidence of bradykinin release syndrome. Alternatively, during CKRT inception, the crystalloid priming can be discarded and not reinfused to the patient, while a blood transfusion can be delivered to the patient through another vascular access in order to balance the amount of patient blood that is required to fill the circuit.

Recently, newer machines have been specifically designed or modified for use in neonates, to minimize extracorporeal volume (as low as <10 ml) during CKRT treatment (and to allow for smaller vascular access requirements and blood flows, as described above) [159–163]. These are only briefly mentioned here, as they are described in a dedicated chapter. Only one of these devices was developed specifically for neonates and is currently available and approved for widespread use in some countries: the Cardio-Renal Pediatric Dialysis Emergency Machine (CARPEDIEM) machine (priming volumes as low as 30 ml). Published experience appears encouraging, though more research is needed to determine the extent to which the goals of using a smaller machine (reducing need for transfusions and other complications of high extracorporeal volume; absolute need for PD in very small infants) are being widely achieved [155, 161, 162]. Another device, specifically developed for neonatal CKRT, is the Newcastle Infant Dialysis and Ultrafiltration System (NIDUS; extracorporeal volume reportedly as low as <10 ml); however, this device is currently still undergoing initial trials and evaluation. Another device, the Aquadex machine (originally conceived as a small device for slow continuous ultrafiltration in adult patients), has been described to be successfully used for delivering CKRT in children below 10 kg, by modifying the original machine technology and application [163].

Dialysis and Replacement Fluid Solutions

In most centers, the same solutions are used for dialysis fluid (in CVVHD) and for replacement fluid (CVVH). Several different types of solutions are shown in Table 8; there are many other types of solutions available and Table 8 simply provides an example of the different contents that dialysis and replacement solutions may have. The majority of centers will utilize commercially available physiologic solutions which contain a buffer, and varying amounts of electrolytes (sodium, potassium, magnesium, calcium, phosphorous) and glucose. Previously, most CKRT solutions used lactate as a buffer. However, more recent solutions use bicarbonate as the primary buffer (with small concentrations of lactate). Evidence from adult patients treated with CKRT suggests that lactate-based solutions may cause rising patient lactate level which may lead to inappropriate concern for tissue ischemia, or conversely, lack of investigation for tissue ischemia if the issue is assumed to be caused by the dialysis solution [164]. Bicarbonate buffered solutions can be made by hospital pharmacies, but are also now available from industry sources. Some centers use pharmacy-prepared solutions; however, this may be associated with safety implications due to compounding errors [165]. Thus, a solution composition validation program should exist in centers that opt to use if pharmacy-made solutions for CKRT. Commercially available CKRT solutions may or may not contain calcium. When performing CKRT using citrate-based anticoagulation (described below), non-calcium containing solutions are generally used. However, if using heparin-based anticoagulation, use of calcium-free solutions may lead to profound hypocalcemia. Within 24–48 h of CKRT initiation, it is extremely common to develop hypokalemia and/or hypophosphatemia. Both potassium and phosphate (in the form of sodium or potassium phosphate) may be added to the CKRT solutions. Some commercially available solutions already contain some potassium or phosphate. There is concern about precipitation of phosphate-calcium crystals with the solution bags, particularly if using calcium-containing solutions. In fact, several in vitro and some

Table 8 Examples (non-exhaustive) of some commercially available CKRT dialysis and/or replacement fluid solutions, to demonstrate the different potential solutions contents. (Many other solutions exist from different companies. However, as shown, typically calcium, potassium, and also phosphate content will vary significantly. Also (not very evident in this table), the types of buffers used may vary significantly as well. Items of interest and of specific clinical significance to patients and CKRT delivery are bolded)

(mmol/L)	Prism0Cal®	Prism0Cal® B22	Prismasol® 0	Prismasol® 4	Phoxilium®[a]
Sodium	140	140	140	140	140
Potassium	–	**4**	–	**4**	**4**
Chloride	106	120.5	109.5	113.5	116
Bicarbonate	32	**22**	32	32	30
Lactate	3	3	3	3	–
Calcium	–	–	**1.75**	**1.75**	**1.25**
Phosphate	–	–	–	–	**1.2**
Magnesium	0.5	0.75	0.5	0.5	0.6
Glucose	–	6.1	6.1	6.1	–
Trisodium citrate	–	–	–	–	–
Citric acid	–	–	–	–	–

human adult studies have shown that precipitation at various CKRT solution concentrations is not common [166–168]. Nevertheless, it is wise to limit maximal solution phosphate concentrations to 1.5–2 mmol/l. For patients with severe hypo- or hypernatremia, in whom rapid change in patient sodium concentration could be detrimental, either sodium chloride or sterile water may be added to the dialysis and/or replacement solution bags to tailor the delivered sodium concentration. Great care must be taken to ensure a lack of errors when doing this. When performing CVVHD (and not CVVH), the dialysis solution can be modified to create a 4 or 5% albumin dialysis solution (by adding 25% albumin to the dialysis solution bags), in order to perform "single-pass albumin dialysis." This type of CKRT may be useful in specific conditions (e.g., Wilson's disease to remove copper; to remove some protein-bound drugs in intoxications; in some cases with severe liver disease) [139, 169].

Blood Flow, Solute Clearance, and Ultrafiltration

With CKRT, as opposed to IHD, the blood flow rate has more substantial impact on circuit patency (lower blood flows being associated with a higher risk of circuit clotting), than on solute clearance. Solute clearance in CKRT is largely a function of dialysis and/or replacement fluid flow rates and of the time spent on treatment. Blood flow rate is determined by patient size, hemofilter size, and vascular access. Blood flow rates can range from 2 to 10 ml/kg/min. In general, for most machines, blood flow rates lower than 30–50 ml/min will be associated with significant risk of clotting of the circuit or of the vascular access. As a general rule of thumb, for patients under 10 kg, rates of 30–50 ml/min are used, and in the largest children, rates of 100–200 ml/min are used. Because of the need to maintain circuit patency with minimum blood flows, CKRT blood flow rates are high in neonates, relative to body size (e.g., 50 ml/min in a 5 kg baby is 10 ml/min/kg); thus, when using citrate anticoagulation (citrate rate prescription is based on blood flow rate, described below), citrate load may be relatively high for neonates.

Regarding treatment prescription, or "dose" of solute clearance, no prospective study in the pediatric setting has addressed its impact on patient mortality or other outcomes [170]. Again, solute clearance "dose" is mainly determined by dialysis fluid rate (in CVVHD), replacement fluid rate (in CVVH) or the addition of dialysis plus replacement fluid rate (in CVVHDF). A standard recommended total solute clearance dose is approximately 2 l/h/1.73 m^2; in patients weighing more than 40 kg, this corresponds approximately to an effluent flow rate of 25–35 ml/kg/h, which is the currently recommended prescription in adult patients [1]. At this level of clearance, the majority of metabolic disturbances of AKI will be managed. However, when prescribing CKRT, the dose should be tailored to the patients' clinical needs. For example, in patients with life-threatening hyperkalemia or hyperammonemia, much higher solute clearance can and should be prescribed (e.g., 4 to 8 l/h/1.73 m^2). However, if prescribing very high clearance rates, it is important to be aware of the negative consequences, including clearance of amino acids (i.e., nutrition), medications, other electrolytes (e.g., magnesium) as well as the possibility of dialysis disequilibrium syndrome in patients with very high urea and/or chronic or acute on chronic kidney disease. Citrate anticoagulation will also be more complicated when using very high solute clearance rates, because of clearance of citrate, requiring closer monitoring (monitoring described below).

Ultrafiltration (net fluid removal from the machine, sometimes referred to as netUF) is a crucial goal of CKRT. NetUF should be approached in such a way that fluid removal goals are achieved, while maintaining patient safety and avoiding aggressive ultrafiltration. The net daily negative patient balance (and thus, hourly net negative patient balance) should be discussed and agreed upon between nephrology and ICU teams and should take into account the netUF rate along with patient residual diuresis and all current and expected fluid infusions to the patient in the next 24 h. Moreover, when determining the desired patient net negative balance goals and ultrafiltration, the presence of other fluid outputs (e.g., urine output; nasogastric tube; stool; chest tube) should be considered, to achieve safe fluid removal targets. There is no absolute safe maximum negative hourly net patient fluid removal rate, but rather, empiric guidelines that may be used. Ultimately, decisions on hourly negative balance will be dictated by the patient's hemodynamic stability together with the urgency of requirement for fluid removal. One rule of thumb to avoid overly aggressive fluid removal in CKRT is to avoid targeting a daily net negative balance (in liters) of greater than 3–5% of body weight, with a lower target in patients who are more hemodynamically unstable. Another rule of thumb is to avoid targeting a net hourly negative balance of greater than 1–2 ml/kg/h. The fundamental difference between netUF (the fluid removed from the machine) and fluid balance (the ultimate difference between fluids infused and fluids removed from the patient) should always be kept in mind. Moreover, it may sometimes be necessary to increase vasopressor support in order to achieve net fluid removal goals. These suggestions are but starting points for approaching fluid removal and should be reassessed frequently and whenever the patient's clinical status changes significantly.

Anticoagulation

Anticoagulation of the CKRT circuit is essential to provide the therapy in children. Heparin and citrate are the two most common forms of anticoagulation. Heparin anticoagulation is achieved by continuous infusion of heparin to the CKRT machine circuit, pre-hemofilter and monitoring partial thromboplastin time (PTT), and/or activated clotting time (targeting systemic PTT ~1.5 times normal and/or activated clotting time of 180–220 s). In certain situations (e.g., postoperative, known bleeding risk, or when using higher blood flows), anticoagulation targets may be lower. The side effects of heparin include systemic anticoagulation and the rare occurrence of induction of heparin induced thrombocytopenia. Patients who are deemed to be at very high bleeding risk should not receive heparin. Increasingly, regional (restricted to the CKRT circuit) citrate anticoagulation is being used by many centers, instead of heparin. Citrate anticoagulation is performed by continuous infusion of citrate solution at the access ("arterial") side of the circuit running from the patient to the CKRT machine. Anticoagulation is achieved by binding to and decreasing ionized calcium in the blood, which is required for the activation of the clotting cascade. Once returned into the bloodstream the calcium-citrate complexes are processed in the Krebs cycle (mainly muscles, liver, and generally all body tissues), calcium is released into the blood, and citrate is metabolized into bicarbonate and ATP. Calcium is infused into a separate systemic central venous line or at the return ("venous") portion of the dialysis catheter, to maintain physiologic ionized calcium in the patient replace calcium losses (part of the complexes citrate-calcium are removed through the filter). The potential complications of regional citrate anticoagulation include metabolic alkalosis (especially if used in combination with bicarbonate buffered CKRT replacement/dialysis solutions) and citrate lock. Citrate lock occurs when the delivery of citric acid and citrate exceeds the patient's hepatic clearance and the clearance of citrate through the hemofilter. As a result, citrate concentrations increase in the patient's blood and bind calcium. Citrate lock is identified by decreasing serum ionized calcium in the presence of rising total calcium (a rule of thumb is the ratio of ionized calcium to total calcium in the patient >2.5–2.8 being indicative of citrate accumulation). A metabolic acidosis may also occur with citrate lock. The treatment for citrate lock (which is essentially citrate accumulation) is to either reduce citrate delivery or increase its clearance (or both). Citrate may be discontinued for 1–4 h and then restarted at a lower citrate delivery rate. Temporarily increasing clearance (increasing the dialysis fluid or replacement fluid rate) may also help reduce systemic citrate concentrations, though close attention to electrolyte abnormalities and other dialytic losses (medications, nutrition) should be considered. Reducing the blood flow may also help to decrease citrate delivery, as citrate delivery rate is dependent on blood flow. When alkalosis is present (due to metabolism of citrate by a functioning liver), either 0.9% normal saline may be delivered as a replacement solution via the CKRT machine or, if available, a lower bicarbonate concentration CKRT solution may be used for dialysis and/or replacement solution. Pediatric studies have reported practical and safe citrate anticoagulation protocols [171–173]. Of note, when there is very severe liver disease or significant systemic hypotension with muscle tissue hypoperfusion (e.g., septic shock), the risk for citrate accumulation increased significantly. Moreover, in small babies, citrate accumulation is quite common, because the blood flows required to maintain circuit patency are relatively high for these patients' body sizes; thus, citrate delivery is also relatively high.

Complications

In addition to the CKRT complications noted above, another complication of CKRT is hypothermia, particularly in infants. This can be avoided by the use of heat lamps, dialysis fluid warming and blood tube warmers. Electrolyte disturbances can occur, particularly hypophosphatemia, hypomagnesemia, and hypokalemia, due to dialytic clearance and can be avoided by frequent electrolyte monitoring and appropriate adjustment of dialysis and/or replacement solution composition. Excessive ultrafiltration is a problem that may frequently occur, but can avoided by careful patient monitoring with hourly fluid balance, and frequent reassessment of the day's goals by the pediatric nephrologist. Incorrect dosing of drugs may also occur. In children receiving prolonged citrate anticoagulation, due to chronic calcium binding in the blood, the potential for increased risk of fractures and bone disease has been described [174].

Nutrition

Adequate nutrition in critically ill children with AKI is a component of their treatment, particularly critically ill children, who are at increased risk of developing protein-energy malnutrition. Critically ill patients with severe AKI are considered highly catabolic and the presence of KRT contributes to nutritional losses, especially amino acids and water-soluble vitamins. Similar to what has been found in adults, amino acid losses by standard clearance CKRT can be anywhere from 10 to 20% of the amount provided in the parenteral nutrition, which is associated with the development of negative nitrogen balance [175, 176]. Given the age-associated baseline elevated protein needs of children and the presence of abnormal amino acid synthesis and increased protein catabolism in critical illness and AKI, daily protein intake should be in the order of at least 3 g/kg/day in children treated with CKRT and should be adjusted if higher solute clearance doses are provided.

Drug Dosing

Drug dosing must often be altered for patients with AKI, not only for avoiding kidney injury, but to avoid toxic accumulation of drugs and their metabolites which are excreted by the kidney. When glomerular filtration rate is estimated to be less than 50% of normal, most drugs excreted by the kidney will require modifications in scheduled dosing. For patients receiving CKRT, drug dosing can be quite challenging. However, understanding the size, volume of distribution, and protein binding of the drug (for each of which, if high, leads to less clearance through the hemofilter) can estimate appropriate drug dosing. Moreover, for some drugs, there may be *adsorption* of the drug to the hemofilter, particularly when using convective clearance (i.e., CVVH). This should be considered if a certain drug a patient is receiving is thought to be critical to their treatment. Several excellent recent review articles discuss how to specifically dose different drugs during CKRT or with AKI, based on these biochemical properties [177, 178]; however, in vivo validation of these concepts is minimal and an area of much-needed research [179].

AKI Follow-Up

As discussed above, there is increasing evidence of long-term negative effects of AKI, including CKD or hypertension. The KDIGO guidelines currently recommend that patients who develop AKI should be evaluated at 3 months after discharge to evaluate for recovery from AKI and/or the development of CKD [1]. No studies have specifically determined and/or evaluated the adequate time or frequency for follow-up after an episode of AKI in children. Moreover, different healthcare contexts will be faced with varying resource issues in being able to follow-up all patients who developed AKI. Some resources are available to help guide how to approach long-term follow-up of AKI in children [180–182].

One approach is described in a publication describing an AKI survivor clinic [182]. Children with Stage 2 AKI or worse are identified by the EHR, triggering referral. A clinic staff reviews EHR-triggered referrals and ensures 3, 6, and 12 month follow-up visits for CKD and hypertension screening and treatment, patient and healthcare provider education, and mitigation of future AKI risks. Such a program requires institutional support, multi-team coordination and an EHR to perform AKI "alerts." Another proposed approach [181] is to risk-stratify/select AKI patients who would benefit the most from follow-up; this selection is not solely based on AKI severity, but also on other factors such as non-recovery from AKI before discharge, location of event (intensive care unit *vs.* hospital ward), urinary biomarker data, and/or AKI recurrence.

Another approach is described below. First, when a child who developed AKI during hospitalization is being prepared for discharge, at the very least, an assessment of (a) complete recovery or non-recovery from the AKI episode (i.e., measure SCr) and blood pressure and (b) overall risk for long-term complications of AKI (i.e., risk factors presented in Fig. 1a) should be made. Patients with Stage 2 AKI or worse are more likely to have long-term complications [183]; those without recovery at discharge should also be considered for follow-up. Based on the level of risk and severity of abnormalities, if a dedicated AKI follow-up clinic or outpatient nephrology resources are not available, then the healthcare provider best suited to perform post-AKI follow-up should be identified before discharge, informed about the AKI episode and provided with instructions on what evaluations to perform at follow-up and criteria for referring to nephrology care. An effort should be made to ensure that patients with clear risks for long-term kidney disease, baseline kidney disease, or significant kidney dysfunction or hypertension before discharge are followed by a nephrologist after discharge. The family should also be educated and informed that an AKI episode occurred, in order to promote prevention of future AKI episodes. Families that are aware of the AKI episode and of the concept of kidney protection are more likely to inform future healthcare providers and potentially more likely to encourage them to avoid nephrotoxic medications. As described in the description of the AKI survivor clinic above [182], at the first follow-up visit, the main goals are determining if the underlying AKI and AKI cause has resolved, educating families (e.g., avoiding nephrotoxins; informing primary physicians of the AKI), screening for kidney health risk factors (e.g., nephrotoxins, obesity), promoting health lifestyle habits and evaluating estimated GFR, proteinuria, and blood pressure. Previously healthy children with no risk factors outlined above and complete AKI resolution before discharge should undergo at least urine (for proteinuria) and blood pressure examination at the first follow-up visit. If all parameters are normal and the patient is felt to be low-risk, follow-up kidney health assessment approximately 1 year later, with reassessment every 1 or 2 years, is likely reasonable.

The Acute Dialysis Quality Initiative 22nd conference consensus document described above (for primary and secondary prevention of AKI) also provides guidance to long-term AKI follow-up, though these recommendations were developed for adults [3]. A pediatric-specific approach will likely be developed in the future. They provide a framework for what post-discharge follow-up visits should include in patients who suffered an AKI episode. This framework is described by the acronym "K.A.M.P.S" and is very concordant with the AKI follow-up approaches described above: *Kidney Function:* estimated GFR; proteinuria/albuminuria assessment; other biomarkers or imaging as indicated (to which we add blood pressure assessment); *Advocacy:* patient and caregiver education; communication with care providers; *Medications*: medication reconciliation, review, and management; review and discuss risk benefits of potentially nephrotoxic medication and review medication potentially impacted by kidney function; *Pressure:* ensure patient understands blood pressure goals and targets; discuss fluid status, ideal weight, role of diuretics; *Sick Day Protocols:* educate patients on medications that need monitoring during acute illnesses (e.g., in some patients, holding ace-inhibitor treatment with significant dehydration episodes).

Conclusions

As shown in this chapter, AKI has been increasingly acknowledged internationally as a common and important problem, with potentially long-lasting negative impacts on kidney health and other outcomes. Many initiatives are underway to guide the care of AKI patients, with a major focus on awareness (by patients, healthcare providers, and healthcare systems) as well as on research to improve AKI diagnosis and healthcare delivery, with a goal of improving outcomes [184, 185].

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Nephrotoxins

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Abstract

The excretion of endogenous and exogenous compounds is a fundamental aspect of renal function, which, by necessity, exposes the kidney to high concentrations of these substances and their metabolites. Some of these compounds are “toxic” to the kidney and substances capable of causing renal damage or injury are termed “nephrotoxins.” While naturally occurring nephrotoxins exist and environmental exposures can cause injury, the focus of this chapter is on the pharmaceutical agents used to treat medical disease and its sequelae. While specific, ubiquitously prescribed agents are discussed in detail, equal weight is placed on highlighting core nephrotoxin concepts, illustrating the most commonly seen nephrotoxicity phenotypes, and describing the mechanisms by which these compounds cause injury. This foundation creates the context for discussing approaches to avoid, minimize, and manage the impact of nephrotoxin exposure. Although targeted treatments for nephrotoxic acute kidney injury do not yet exist, broad multidisciplinary strategies are capable of predicting, preventing, and mitigating the impact of these medications. Furthermore, a thorough understanding of the core concepts underpinning nephrotoxicity will allow future development of disease-specific interventions.

Keywords

Nephrotoxin · Nephrotoxic · Acute kidney injury · AKI · Prevention

Introduction

One of the most fundamental aspects of kidney function is the elimination of endogenous and exogenous compounds. Indeed, evaluation of excretory function is the cornerstone of assessing renal physiologic capacity. Excretion, by necessity, exposes the kidney to high concentrations of these substances; at high concentrations, these compounds and their metabolites can be toxic, causing injury or damage to the kidneys [1–4]. In 1965, Dr. George Schreiner described this “toxin nephropathy” as “any adverse functional or structural changes in the kidney due to the effect of a chemical or biological product that is inhaled, ingested, injected, or otherwise absorbed, or that yields toxic metabolites with an identifiable adverse effect to the kidneys [5, 6].” Since then, this “nephrotoxicity” has been variably referred to in the literature as “drug-associated acute renal failure” [7], “drug-associated or drug-induced acute kidney injury” [8], “drug-induced kidney disease” [9], or occasionally with disease-subtype-specific language such as “drug-induced ANCA-associated vasculitis” [10]. However, the concept of nephrotoxicity has evolved and now broadly refers to any adverse effect of a substance on kidney structure or function.

As a corollary, substances capable of causing such injury are now referred to as nephrotoxins.

Over the past two decades, nephrotoxins and nephrotoxicity have been widely studied and our understanding of mechanisms, epidemiology, and management has increased substantially. In this context, it is important to introduce several broad concepts. The first such concept is the manner in which the toxicity presents itself; recognizing toxicity phenotypes is central to our understanding of the agents themselves. In 2015, Mehta characterized nephrotoxicity as having four distinct phenotypes [9]. Specific mechanisms of injury are discussed later in the chapter; however, in terms of clinical presentation of disease, this is an informative construct. The most ubiquitous and widely accepted phenotype is that of acute kidney injury (AKI). In these situations, after receiving a nephrotoxin, a patient experiences a rise in serum creatinine consistent with injury and reduced excretory function; aminoglycosides are a classic example of the AKI phenotype [11]. A second phenotype that is common is crystalluria. These patients experience crystal formation in the urinary tubules or, less commonly, nephrolithiasis; acyclovir is a nephrotoxin which commonly presents as crystalluria and sulfa-based antibiotics can lead to stone formation [9, 12, 13]. A third phenotype is that of tubular dysfunction. Such patients present with preserved excretory function but have evidence of tubular mishandling of fluid and/or electrolytes; the Fanconi syndrome seen with ifosfamide and the diabetes insipidus associated with lithium therapy are classic examples of this phenomenon [14–17]. The fourth phenotype described is glomerular disease. While less common than the other three phenotypes, these patients may present with hematuria, proteinuria, or even a mixed nephritic/nephrotic picture. Examples include the nephrotic syndrome and podocyte dysfunction seen with sirolimus, the focal segmental glomerulosclerosis seen with pamidronate or adriamycin, and the anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis seen with d-penicillamine [18–20].

The second important concept is the temporal component of nephrotoxicity; renal injury generally occurs over three time horizons – acute, subacute, or chronic [9]. Acute nephrotoxicity characterizes injury, which occurs within approximately 1 week of exposure; the acute tubular necrosis (ATN) caused by amphotericin is a good example of acute nephrotoxicity [21]. Subacute nephrotoxicity, such as the acute interstitial nephritis (AIN) seen with nonsteroidal anti-inflammatory drug (NSAID) exposure, typically develops over weeks to months [22, 23]. Finally, chronic nephrotoxicity is commonly defined as renal disease seen after at least 3 months of exposure; the nephropathy seen with calcineurin inhibitor administration often becomes apparent months to years after solid organ transplantation [24]. Knowledge of the nephrotoxin time horizon for injury helps set expectations and guide monitoring strategies.

Third, it is important to consider the exposure-response relationship of a nephrotoxin as this may influence management and mitigation decisions. Certain medications or classes exhibit a dose-dependent effect [25], described as a “Type A” nephrotoxicity [9]. Drug toxicity in these cases is typically a function of the maximal concentration achieved or the overall exposure as described by the area under the concentration-time curve [25]. While drug discontinuation is the preferred intervention to limit further kidney damage, a decrease in the drug dose or rate of administration may be a suitable alternative option if the drug is required and no other therapies are available. Other medications or classes are associated with an idiosyncratic or unpredictable effect, known as “Type B” nephrotoxicity [9]. Kidney injury in these situations is dose-independent and, as a result, a dose reduction is unlikely to affect the potential for long-term kidney damage; discontinuation of the substance is typically necessary to avoid further injury.

A fourth consideration, specific to children, is the influence of developmental factors on the risk for toxicity. In neonates, the immaturity of tubular transporters in early life may act as a renal protective mechanism for drugs that increase their potential for injury via intracellular concentration (e.g., aminoglycosides) [26, 27]. On the other hand, neonates’ inability to maximally concentrate their urine may predispose to intravascular volume depletion which increases the nephrotoxic potential of medications that affect renal hemodynamics [27–30]. In infants, renal excretory function gradually increases from birth, reaching adult normative values at approximately 2 years of age

[31]. During this transition, accurate dosing can be challenging and the risk of nephrotoxicity may be increased, particularly with substances whose excretion is primarily renal in nature [32]. Other pharmacokinetic changes in the first 2 years of life, including the evolution of enzymatic function and changes to the proportion of total body water, can alter drug distribution, overall exposure, and toxicity risk [27].

Finally, it is important to highlight the concept of comprehensive "nephrotoxin burden." While many epidemiologic studies have investigated the association between a specific substance in a specific population (e.g., cisplatin in pediatric cancer patients or aminoglycosides in patients with cystic fibrosis) [33, 34], the overall nephrotoxic burden a patient is exposed to may have more clinical relevance than the impact of any single agent. This concept was first introduced by Merlin Thomas in 2000 when he coined the term, "triple whammy" [35], which referred to the combination of a diuretic, an NSAID, and an angiotensin-converting enzyme (ACE) inhibitor ubiquitously prescribed to patients with heart failure. He found that more than 50% of iatrogenic AKI in this population could be ascribed to one or more of these agents [6, 35]. The concept of nephrotoxic burden has been further refined by the Nephrotoxic Injury Negated by Just-in-time Action (NINJA) program [36–39]. This initiative defines a large nephrotoxic burden as receipt of three or more agents concomitantly or the administration of particularly injurious agents for a prolonged duration. Studies designed to identify patients exposed to a large nephrotoxin burden have proven quite successful at mitigating injury [36].

In summary, our conceptual understanding of nephrotoxins, the injury they cause, and optimal management strategies have grown tremendously. Although specific treatments for nephrotoxic injury are not currently available, approaches designed to increase awareness, identify high-risk patients, and prevent unnecessary exposure have helped to mitigate disease severity. This chapter will focus on nephrotoxic compounds, mechanisms of injury, and management strategies. Additionally, some specific, commonly used nephrotoxins will be described in detail. Given the continued developments in medicine, the globalization of society, as well as the changes in our environment, the risk for injury is not likely to decrease over time and a robust understanding of this topic will be of paramount importance for practitioners of pediatric nephrology.

Types of Nephrotoxins

The term nephrotoxin is generally used to refer to pharmaceuticals that are intentionally administered despite having a side effect profile that includes kidney injury. However, it is important to be aware of the numerous other substances capable of causing significant kidney harm which include both synthetic and naturally occurring toxins. Exposure to some of these various nephrotoxic substances may depend on geographical location, seasonality, and specific subpopulation.

Pharmaceuticals

Pharmaceuticals have been the subject of extensive research and inquiry as many drugs have a side effect profile containing the undesirable potential for kidney injury. These medications may be prescription or over-the-counter (OTC). Nonprescription medications, while widely available, are not without risk [6, 22, 40, 41]; indeed, The 2012 Kidney Disease Improving Global Outcomes (KDIGO) AKI guidelines underscore the importance of considering oral nephrotoxins such as NSAIDs, in addition to the more commonly considered intravenous medications, when performing risk stratification and prediction modeling [42]. Medications which have only recently become available likely warrant more deliberate reflection before drawing firm conclusions. Vascular endothelial growth factor (VEGF) inhibitors, for example, have mixed data with regard to renal injury; ultimately, their true nephrotoxic potential will be born out over time [43]. While it is challenging to produce an all-encompassing list of nephrotoxic pharmaceutical agents, an article by Perazella and the NINJA collaborative have attempted to do just

Table 1 Nephrotoxic medications and compounds by category or class

Pharmaceutical agents	Pharmaceutical agents (cont.)	Naturally occurring compounds
ACE inhibitors (category)	NSAIDs (category)	Aristolochic acid
Aminoglycosides (category)	Orlistat	Cape aloes
Analgesic combinations (category)	Pentostatin	Datura species
Anti-angiogenesis medications (category)	Phenacetin	Dichromate
Antivirals (category)	Platins (category)	Ephedra species
Amphotericin B	Polymixin B	Glycyrrhiza species
ARBs (category)	Selective COX-2 inhibitors (category)	Mefenamic acid
Bisphosphonates (category)	Sucrose	Melamine
Calcineurin inhibitors (category)	SGLT-2 inhibitors (category)	Phenylbutazone
Colistin	Statins (category)	Taxus celebica
Dextran	Sulfadiazine	Uno delgatta
Gemcitibine	Quinolones (category)	
Hydroxyethyl starch	Topiramate	***Environmental compounds***
Ifosfamide	Vancomycin	Bismuth
Immunotherapies	Zonisamide	Cadmium
Interleukin-2		Copper
Mannitol		Germanium
Mesalamine		Hydrocarbons
Methotrexate		Lead
Methoxyflurane		Mercury
Mitomicin		Silicon
mTOR inhibitors (category)		Uranium

This comprenensive list of nephrotoxins was adapted from Perazella MA and includes medications and compounds likely to be encountered by patients in the inpatient and ambulatory settings [44]

ACE angiotensin-converting enzyme, *ARB* angiotensin II receptor blocker, *NSAID* nonsteroidal anti-inflammatory drug, *COX* cyclooxygenase

that [36, 38, 44]. Table 1 provides a general list of nephrotoxic compounds, grouped by therapeutic category; this table is based on the work of Perazella and contains a comprehesive list of pharmaceuticals as well as the natural compunds and environmental toxins described later. The NINJA list of nephrotoxins focuses specifically on medications prescribed to hospitalized children. This list is subject to evidence-based review and refinement [39]; Table 2 provides the most current list alphabetically [36].

Naturally Occurring Nephrotoxins

One category of naturally occurring nephrotoxins which are of great importance from a global health standpoint are animal and insect venoms. For example, the brown recluse spider (*Loxosceles reclusa*), which is endemic to the Southcentral United States, is well known for the toxicity of its bite [45]; there are more than 100 species of *Loxosceles* spiders and systemic loxoscelism is common throughout South America [46]. In the tropical and sub-tropical regions of the world, venomous snakes account for millions of bites annually, with significant morbidity and mortality [47]. Scorpion envenomation is more common in parts of Central and South America, Northern Africa, and the Middle East [48]. Envenomation due to contact with the bristles of the *Lonomia obliqua* caterpillar has been seen with increasing frequency, possibly due to environmental changes in Brazil [49]. The toxicity of Hymenoptera (bees, wasps, ants) stings may result from hundreds of stings or as few as 20. Despite the existence of >150,000 species of Hymenoptera, generally only envenomation from the honeybee, yellow jacket, and giant hornet are considered clinically relevant from a nephrotoxcity standpoint

Table 2 Nephrotoxins Monitored by NINJA Initiative

Acyclovir	Gentamicin*	Naproxen
Ambisome	Ibuprofen	Pamidronate disodium
Amikacin*	Ifosfamide	Pentamidine
Amphotericin B	Indomethacin	Piperacillin
Aspirin	Iodixanol (Visipaque)	Piperacillin/Tazobactam
Captopril	Iohexol (Omnipaque)	Polymixin B
Carboplatin	Iopamidol (Isovue)	Sirolimus
Celecoxib	Iopromide	Sulfasalazine
Cidofovir	Ioversol	Tacrolimus
Cisplatin	Ioxaglate meglumine/sodium	Tenofovir
Colistimethate	Ioxilan	Ticarcillin/clavulanic acid
Cyclosporine	Ketorolac	Tobramycin*
Deferasirox	Lisinopril	Topiramate
Diatrizoate meglumine	Lithium	Valacyclovir
Diatrizoate sodium	Losartan	Valganciclovir
Enalapril	Mesalamine	Valsartan
Enalaprilat	Methotrexate	Vancomycin*
Foscarnet	Mitomycin	Zoledronic acid
Ganciclovir	Nafcillin	Zonisamide

This is the curated list of medications administered to hospitalized children curated by the NINJA collaborative. Exposure constitutes the concomitant administration of 3 of these 57 agents or the administration of highly nephrotoxic agents (*) for an extended period of time (greater than or equal to 3 days). This represents the current list as of December 31st 2020

[50]. Renal injury from envenomations can be due to a variety of mechanisms including hemodynamic changes, microvascular thrombosis, AIN or direct cellular toxicity.

Botanicals are another naturally occurring source of nephrotoxicity; these compounds cause renal injury by intentional consumption as a food source (often to excess) or as a part of traditional healing methods. For example, excessive ingestion of star fruit [51], rhubarb [52], cashew pseudofruit [53], or djenkol beans [54] can cause acute crystal nephropathy. Traditional Chinese medicines may not be solitary substances, but rather complicated compounds whose toxicity is due to the presence of aristolochic acids, alkaloids, flavonoids, and/or anthraquinones [55]. Aristolochic acids in particular are not limited to Chinese therapies as products containing this compound are consumed around the world to treat a variety of conditions and have been linked to interstitial nephritis and renal failure [56]. The castor bean plant, infamous due to its derivative ricin, has also long been used as phytotherapy by many cultures [57]. A renewed interest in mycology has spurned an increase in *Amanita phalloides* (poison mushroom) ingestion with most cases being accidental due to misidentification [58]. These plant toxins, once ingested, cause cellular apoptosis, necrosis, and microvascular thrombosis [59, 60].

Environmental Nephrotoxins

Environmental hazards where humans routinely live, work, and play can pose a daily threat and have been linked to millions of deaths [61]. Ethylene glycol poisoning from antifreeze ingestion has received significant attention, with campaigns focused on decreasing exposure and mitigating the adverse effects including crystalluria [62]. Heavy metal poisoning, such as that that seen with lead, can occur as an occupational exposure. Alternatively, although nonoccupational exposures were thought to have been addressed decades ago by eliminating lead-based paint, pipes, and gasoline, such exposures continue to this day; the recent water crisis in Flint, Michigan (Unites States) is a reminder of this fact [63]. Outside of the United

States, currently less than 50% of countries have legal restrictions in place to limit lead-based paints; furthermore, exposure through contaminated ground water, traditional medicines, and soil remains a global health concern. Other examples of heavy metal nephrotoxins include the cadmium and mercury found in fuel combustion and some foods; uranium and arsenic have been detected in contaminated soil and water. Exposure to these heavy metals can cause tubular dysfunction acutely and progressive chronic kidney disease (CKD) with ongoing exposure [61]. Finally, there is a strong link between residing in agricultural communities and kidney disease [64]. The etiology is likely multifactorial, however, pesticides are known to have numerous adverse effects in humans [65]. Unfortunately, determining which specific agricultural chemicals are nephrotoxic is challenging as comunities are often exposed to multiple pesticides concurrently [61]. There are a number of drugs of abuse, such as inhaled solvents, heroin, and cocaine which are known to have nephrotoxic potential. The toxicity of synthetic cannabinoids [66], synthetic cathionones, and 3,4-methylenedioxymethamphetamine (MDMA, ectasy) are well described but may vary as unique clusters of injury imply a shifting composition of the products [67]. More recent data suggests anabolic steroid use may be associated with CKD as well [68].

Mechanisms of Nephrotoxicity

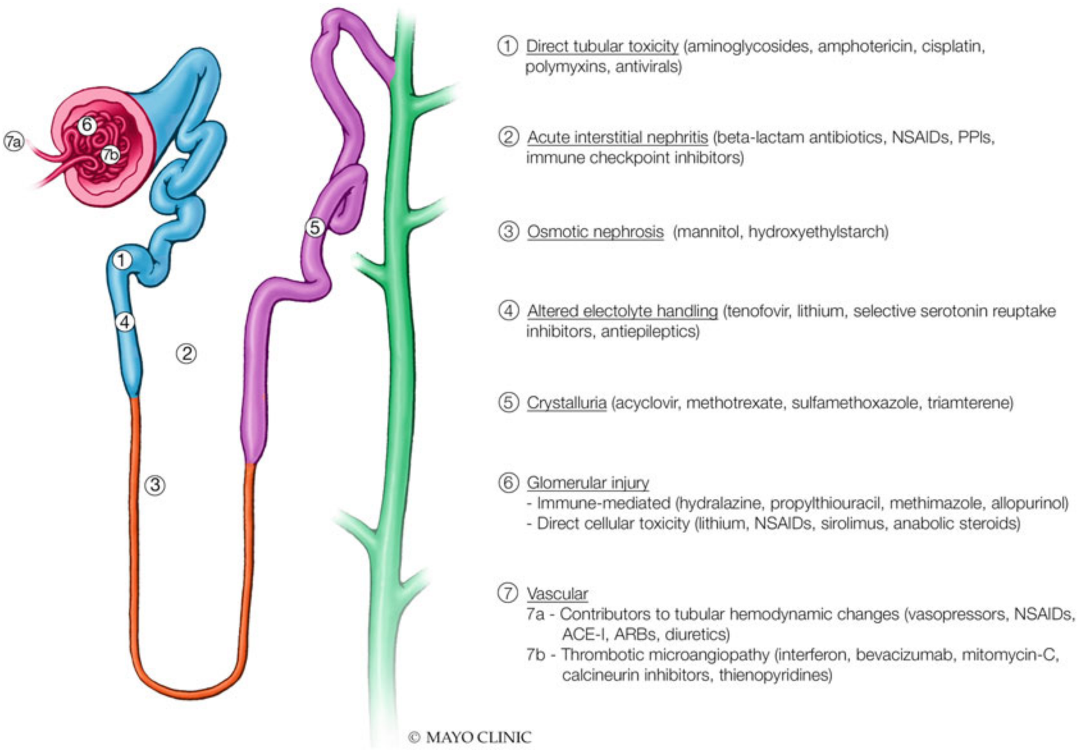
One of the most important approaches used to categorize and catalog nephrotoxins is based upon the mechanism of toxicity (Fig. 1). Understanding the manner by which injury occurs helps providers prevent, mitigate, and potentially treat nephrotoxicity. For example, the use of multiple medications with comparable mechanisms of injury may lead to a synergistic risk for complications [69]. Alternatively, if one knows that an agent causes kidney damage via urinary crystal formation, increasing urine output and diluting the concentration of the drug may help reduce injury. Furthermore, although no treatments exist for nephrotoxicity once it occurs, an understanding of the underlying mechanisms of injury will allow for the development of targeted therapeutics in the future. It is important to note that while certain drugs and their metabolites classically present with one specific mechanism of injury, many will present variably. For example, vancomycin-associated nephrotoxicity may manifest as ATN, AIN, or cast nephropathy [70–72]; similarly, NSAIDs may cause kidney injury via a number of different pathways. Finally, the ultimate presentation of injury is in many respects dependent on patient factors and clinical context.

Pseudo-nephrotoxicity

Nephrotoxicity results from extrinsic or intrinsic deleterious effects of a toxin on kidney structure or function. At the bedside, one of the early warning signs is a rise in the endogenous serum biomarker creatinine. Serum creatinine is a freely filtered small molecule that exhibits an inverse relationship with kidney function. A fraction of serum creatinine is also eliminated through active tubular secretion via several transporters including the organic cation transporter 2 (OCT2) [73]. The drug trimethoprim is an OCT2 inhibitor which inhibits tubular secretion of creatinine, increasing serum creatinine concentrations without directly damaging the kidney [74]. This is an example of “pseudo-nephrotoxicity.”

Indirect Nephrotoxicity

In certain cases, medications may not be directly nephrotoxic but rather they contribute to systemic disease which, in turn, causes kidney damage. Rhabdomyolysis is an example of a systemic syndrome which results in indirect nephrotoxicity [75]. Several mechanisms contribute to AKI associated with rhabdomyolysis including vasoconstriction, direct ischemic injury, proliferation of free radicals, and tubular obstruction associated with myoglobinuria [76]. Hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors or “statins” are not classically thought of as nephrotoxins. However, when used in



① Direct tubular toxicity (aminoglycosides, amphotericin, cisplatin, polymyxins, antivirals)

② Acute interstitial nephritis (beta-lactam antibiotics, NSAIDs, PPIs, immune checkpoint inhibitors)

③ Osmotic nephrosis (mannitol, hydroxyethylstarch)

④ Altered electolyte handling (tenofovir, lithium, selective serotonin reuptake inhibitors, antiepileptics)

⑤ Crystalluria (acyclovir, methotrexate, sulfamethoxazole, triamterene)

⑥ Glomerular injury
- Immune-mediated (hydralazine, propylthiouracil, methimazole, allopurinol)
- Direct cellular toxicity (lithium, NSAIDs, sirolimus, anabolic steroids)

⑦ Vascular
7a - Contributors to tubular hemodynamic changes (vasopressors, NSAIDs, ACE-I, ARBs, diuretics)
7b - Thrombotic microangiopathy (interferon, bevacizumab, mitomycin-C, calcineurin inhibitors, thienopyridines)

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Fig. 1 Mechanisms of Nephrotoxic Injury. The primary mechanisms by which nephrotoxins cause kidney injury are highlighted along the length of the nephron segement. Some agents, such as aminoglycosides directly injure renal tubules (1). Others, such as some beta-lactam antibiotics, induce an acute interstitial nephritis (2). Medications such as mannitol may cause osmotic nephrosis which is marked by tubular swelling and death (3). Many substances have minimal impact on filtration but instead disturb fluid and electolyte homeostasis (4). Acyclovir and other agents which are insoluble in urine may precipitate in the tubules causing cell injury and obstruction to urinary flow (5). Although uncommon, specific pharmaceuticals can cause immune-mediatied glomerular injury or direct damage to podoctes (6). Finally, many medications adversely affect intrarenal hemodymanics (7a) or renal blood flow (7b). NSAID nonsteroidal anti-inflammatory durg, PPI proton pump inhibitor, ACE-I angiotensin-converting enzyme inhibitor, ARB angiotensin II receptor blockers. (Used with permission of Mayo Foundation for Medical Education and Research, all rights reserved)

settings where the risk for rhabdomyolysis is heightened, such as in combination with a macrolide antibiotic or in a patient with a genetic variant at the *SLCO1B1* transporter, their use can lead to kidney damage [69, 77, 78].

Vascular Complications

The kidney receives approximately 25% of the cardiac output. It is therefore sensitive to factors that influence systemic hemodynamics. Excessive exogenous fluid administration may increase venous pressure in the kidney, leading to interstitial edema which can reduce renal blood flow, glomerular filtration rate (GFR), and lead to additional salt and water retention [79]; indeed, fluid overload in critically ill children has been associated with major adverse kidney events and poorer outcomes [80, 81]. The opposite effect, iatrogenic intravascular volume depletion due to excessive diuresis, may decrease effective circulating arterial blood volume and compromise kidney perfusion. Drugs that lead to vasoconstriction or alter transglomerular pressure can also contribute to this phenomenon. Vasopressors such as norepinephrine may increase the risk for AKI due to renal vasoconstriction [8]; alternatively, vasopressors may mitigate systemic hypotension and restore effective kidney perfusion [82]. Similarly, calcineurin inhibitors lead to dose-dependent vasoconstriction of the afferent and efferent arterioles [83, 84]. Renin-angiotensin-aldosterone system (RAAS) inhibitors (e.g., ACE inhibitors and angiotensin II receptor blockers or ARBs) decrease the vasoconstrictive effect of angiotensin II at the efferent arteriole and reduce glomerular filtration pressure. Similarly, NSAIDs inhibit prostaglandin synthesis which decreases afferent arteriolar vasodilation with a comparable effect on glomerular pressures and GFR [23]. The hemodynamic effects of these medications are especially problematic in patients with reduced baseline kidney perfusion pressures such as those with shock or preexisting intravascular volume depletion. Perturbation of the vasoconstriction/vasodilatation balance may promote ischemia and loss of GFR, however, in many situations, the decrease in GFR does not necessarily reflect cellular damage. It is also important to note that some medications cause endothelial injury which manifests as thrombotic microangiopathy (TMA). Drug-associated TMA may be seen with anti-angiogenic drugs, chemotherapeutics, calcineurin inhibitors, interferon, thienopyridines, and quinine [85–89].

Glomerular Disease

Several drugs are associated with immune-mediated glomerular disease through production of autoantibodies. Manifestations include ANCA-associated vasculitis with necrotizing, crescentic, pauci-immune glomerulonephritis, drug-induced lupus, and drug-associated membranous nephropathy; common examples of drugs that may lead to immune-mediated glomerular diseases include hydralazine, propylthiouracil, methimazole, cocaine, allopurinol, and procainamide [10, 90]. Drugs may also cause glomerular disease via direct cellular toxicity at the level of the podocyte, the endothelial cell, or the mesangial cell; drug-induced podocytopathies include the minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) associated with interferon, anabolic steroids, bisphosphonates, and sirolimus [90–92]. Lithium and NSAIDs may also lead to podocytopathies, but these are not the most common manifestations of nephrotoxicity with these drugs.

Direct Tubular Toxicity

Drugs or their metabolites may directly damage tubular cells via mitochondrial injury, oxidative stress, or DNA damage that can lead to apoptosis and necrosis [71]. Cisplatin, an antineoplastic medication, is well known to be associated with this pattern of injury. Cisplatin is freely filtered at the glomerulus, reabsorbed into proximal tubular cells via active transport, and secreted into tubular cells by the basolateral OCT2. While in tubular epithelial cells, the parent drug and a nephrotoxic metabolite lead to direct cellular damage [84, 93]. Aminoglycosides (e.g., gentamicin, amikacin) undergo glomerular filtration, endocytosis into tubular cells, and intracellular accumulation which can trigger an injury cascade resulting in cell death [71]. Other medications known to cause direct tubular toxicity include amphotericin deoxycholate, polymyxins, cidofovir, adefovir, foscarnet, tenofovir, and radiocontrast agents [25, 71, 84].

Acute Interstitial Nephritis

Drug-associated AIN is thought to be a cell-mediated immune response, analogous to a type 4 (delayed type) hypersensitivity reaction [94]. In AIN, drugs may act as haptens to modify the endogenous response to native renal proteins or induce an autoimmune reaction to the tubular basement membrane through molecular mimicry [95]. Drugs may also trigger systemic immune-activation that leads to deposition or sequestration of immune complexes in the kidney interstitium [94, 96]. Interstitial infiltration by lymphocytes, macrophages, eosinophils, and mast cells leads to inflammation and eventually fibrosis [71]. On kidney biopsies, the primary pathologic findings include interstitial inflammation, edema, and tubulitis [90]. Drugs and drug classes associated with AIN include beta-lactam antibiotics, rifampin, sulfonamides, NSAIDs, proton pump inhibitors, and immune checkpoint inhibitors [95].

Osmotic Nephrosis

Drugs such as mannitol, hydroxyethyl starch, immunoglobulins (predominantly those containing sucrose), and dextrans may lead to osmotic nephrosis. In these cases, drug molecules undergo pinocytosis, cellular accumulation, leading to cells swelling, vacuolation, and obstruction of the tubular lumen [71].

Crystalluria/Nephrolithiasis

Certain medications or their metabolites are insoluble in the urine and can result in crystalline nephropathy and renal calculi [9, 71]. Crystals typically form in the distal aspects of the tubules, obstruct urine flow, and lead to injury of the surrounding kidney tissue. Crystal formation is typically exacerbated by volume depletion, reduced urinary flow rates, high drug doses, rapid drug infusion rates, and altered urine pH. Drugs causing crystalline nephropathy include acyclovir, methotrexate, sulfamethoxazole, indinavir, atazanvir, triamterene, ciprofloxacin, and ethylene glycol [71]. Systemic syndromes may also lead to crystal deposition in the kidney. One such example is the tumor lysis syndrome. Systemic chemotherapy for a rapidly proliferating hematologic malignancy may trigger hyperuricemia, metabolic acidosis, and an increase in the calcium-phosphorous product. A net consequence of these systemic effects is AKI from inflammation, crystal deposition, renal vasoconstriction, and impaired autoregulation [97].

Altered Electrolyte Handling

Drugs may alter how the kidney handles phosphate, glucose, magnesium, potassium, sodium, or water [9]. Specific patterns may include the syndrome of inappropriate antidiuretic hormone (SIADH), diabetes insipidus, or an acquired Fanconi syndrome. Drugs causing this type of kidney dysfunction include lithium, tenofovir, ifosfamide, selective-serotonin reuptake inhibitors, anti-epileptics, and vincristine [98].

Specific Nephrotoxic Medications

While a comprehensive discussion of every nephrotoxin is beyond the scope of this chapter, an in-depth discussion of several injurious substances will help underscore many of the general concepts described here within. Additionally, these medications will serve as the basis for the discussions around prevention, mitigation, and treatment of nephrotoxicity which are laid out later in the chapter. As such, this section will focus on pharmaceutical agents that are commonly used, extensively studied, have a well understood mechanism of injury, or an effectively described epidemiology; particular attention was paid to medications presenting with the AKI phenotype.

Vancomycin

Vancomycin is a glycopeptide antibiotic which inhibits cell wall formation. Although clinically introduced in 1958, nephrotoxicity limited its use which was “reserved for the treatment of potentially life threatening infections which cannot be treated with less toxic antibiotics” [99, 100]. However, antibiotic resistance to methicillin prompted increasing usage as the purported benefits began to outweigh concerns about toxicity [101, 102]. Since then, vancomycin usage has increased and it is now one of the most commonly prescribed antibiotics for treatment of gram-positive organisms [103]. Although it can be given orally or intravenously, most of its nephrotoxicity has been ascribed to intravenous (IV) administration, which will be the focus of this section.

Most of our mechanistic understanding of vancomycin-associated nephrotoxicity is from animal models. These data suggest that vancomycin accumulates in the proximal tubule cells, specifically in the lysosomes [104]. Once present, vancomycin causes oxidative stress, autophagy, and apoptosis [100, 104]. The nephrotoxic phenotype of vancomycin is AKI; compared with patients receiving a non-glycopeptide antibiotic, those prescribed vancomycin experience a relative risk for AKI of 2.45 (95th CI 1.69–3.55) [105]. Although the incidence of AKI in patients receiving vancomycin seems to be highly dependent on drug and patient factors, the incidence ranges from 5% to 43% [106]. Patient-related risk factors include obesity, CKD, severity of illness, and concurrent administration of other nephrotoxic agents [100]. Obesity may increase AKI risk in patients receiving vancomycin due to a discrepancy between actual body weight and ideal body weight; use of a more dosing-appropriate weight and drug exposure monitoring can mitigate this risk [107, 108]. Patients with preexisting CKD are at increased risk for developing vancomycin-associated nephrotoxicity, primarily since clearance is directly related to GFR. Patients with lower GFRs clear vancomycin less effectively, increasing systemic exposure and potential for injury; the lack of excellent dosing data in patients with CKD exacerbates this risk [109]. Similarly, though the causal relationship is poorly understood, patients requiring critical care have increased risk for vancomycin-associated injury compared to those receiving acute care [110]. Finally, vancomycin nephrotoxicity risk is increased by coadministration of other known nephrotoxic agents which is relatively unsurprising given our understanding of nephrotoxic burden. However, one particular interaction warrants additional discussion. As the bactericidal effect of vancomycin is isolated primarily to gram-positive organisms, when an infectious agent has not been identified and broad coverage is required, it is frequently coadministered alongside an agent with potent gram-negative coverage. Notably, the combination of vancomycin and piperacillin-tazobactam has been associated with particularly increased AKI risk. In one single center study, administration of vancomycin and piperacillin-tazobactam was associated with a 6.8-fold (95th CI 1.5–30.9) increased risk for AKI compared with co-administration of vancomycin and meropenem [111]. In a meta-analysis of six studies in adults, the combination of these two antibiotics was associated with an increased risk compared to administration of vancomycin alone (OR 2.77, 95th CI 1.94–3.96) [112]. A systematic review found a similar association but highlighted the fact that the majority of studies are small, retrospective, and unadjusted [113]. This association has been described specifically in pediatric populations as well; a meta-analysis of studies involving children found increased AKI risk with concomitant vancomycin and piperacillin-tazobactam administration [114].

Drug-related risk factors include trough levels or area under the curve (AUC) determinations (dose/exposure proxy), duration of therapy, and method of administration [100]. The best available data suggests that in vivo drug exposure strongly affects vancomycin nephrotoxicity risk [100, 106]. The most commonly used method of assessing systemic exposure is monitoring of trough concentrations. Levels higher than 15 mg/L have been associated with higher AKI risk, which is potentially problematic given historical recommendation to target trough levels of 15–20 mg/L in severe infections in order to insure adequate AUC to minimum inhibitory concentration (MIC) ratios. In children, data suggest that lower trough levels are adequate to achieve the required AUC; however, current guidelines recommend using AUC rather than trough level monitoring whenever possible [115, 116]. Regardless of the method used to determine exposure, higher exposure increases AKI risk. Another way to measure exposure is duration of therapy. One meta-analysis found that courses of 7–14 days duration were associated with higher AKI risk; indeed, most AKI events occurred after 7 days of therapy [106]. Finally, there is some data that continuous infusions of vancomycin decrease nephrotoxicity risk compared to intermittently dosed regimens [100, 115].

Aminoglycosides

Although several aminoglycosides exist, the most commonly prescribed are amikacin, gentamicin, and tobramycin. Typically, they are used to treat infections with aerobic, gram-negative bacteria; however, they have some gram-positive activity as well. Aminoglycosides, which were introduced in the 1940s, prevent protein synthesis by binding to ribosomal subunits [25]. They are freely filtered at the glomerulus and accumulate in proximal tubular cells; elevated drug concentrations cause injury via a direct cytotoxic effect [25]. The nephrotoxic phenotype of aminoglycosides is AKI. Studies in adults and children suggest that AKI rates can be as high as 20–33% [11, 25, 117, 118]. Although data suggest that severity of illness and comorbid conditions increase AKI risk, studies using modern definitions of AKI suggest that at least 20% of noncritically ill children receiving aminoglycosides for more than 5 days will develop AKI [11, 25, 117]. Aminoglycoside-associated AKI develops 5–7 days after initiation and, unlike many other causes of AKI, tends to be non-oliguric [11]; data suggest that aminoglycosides may damage the distal tubule and collecting duct in addition to the proximal tubule, thereby inhibiting urinary concentration [119, 120]. In addition to AKI, patients with aminoglycoside nephrotoxicity can develop hypokalemia, hypophosphatemia, hypomagnesemia, and even a Fanconi-like phenomenon [11, 121]; these findings are likely reflective of the tubular damage the drug causes.

As is the case with vancomycin, there are patient and treatment-specific risk factors for aminoglycoside-associated nephrotoxicity. With regard to patient-related risks, advancing age has been shown to increase risk, however, this phenomenon is exclusive to elderly populations and is not relevant to pediatric medicine [122]; the AKI risk seems similar from neonates to adolescents and young adults [11]. Additional risk factors include underlying severity of illness and comorbid disease; patients with sepsis, CKD, liver failure, and leukemia have been described as at increased risk [11, 121, 123, 124]. Interestingly, it is not clear whether CKD is a causative risk factor or that the reduced GFR in these patients increases the risk for overdosing the medication. Reduced effective circulating volume and lower kidney perfusion pressures may increase AKI risk in patients receiving aminoglycosides [122, 125, 126]. Finally, the concomitant administration of other medications, especially nephrotoxins, increases risk [11, 121, 127].

Beyond patient-specific risk factors, there are some risk factors specific to the administration of the aminoglycoside itself. One such risk is the duration of therapy. As stated above, aminoglycoside-associated AKI is less common when courses are shorter than 5–7 days; longer courses increase exposure, proximal tubular accumulation, and nephrotoxicity [11, 121]. Another factor is the drug level; while AKI may occur with medication levels within the target therapeutic range, higher levels increase this risk in most studies [128]. Interestingly, many have recommended giving a larger dose of aminoglycosides once a day, rather than smaller doses at scheduled intervals throughout the day. While data in animal studies suggested a lower risk of nephrotoxicity, this has not been borne out clinically in most studies and meta-analyses [129, 130]. There does, however, remain a theoretical benefit and once daily dosing is no less effective. The method of administration also affects risk. While AKI has been described with inhaled aminoglycosides, the risk seems to be substantially lower than that of IV administered agents [131, 132]. Finally, the choice of aminoglycoside influences AKI risk and the nephrotoxicity of the agents is not the same at equipotent doses. Although the data is somewhat mixed, the nephrotoxicity of amikacin is less than tobramycin which, in turn, is less than gentamicin [121].

Intravenous Contrast

Iodinated contrast media has long been considered a nephrotoxin. The pathogenesis of kidney injury-related to administration of these agents is incompletely understood. The best data available suggest that at least two primary mechanisms are responsible [133, 134]. First, contrast media, perhaps via increased release of endothelin or adenosine (vasoconstrictors) and inhibition of prostaglandins and nitric oxide (vasodilators), causes renal vasoconstriction and ischemic injury [133, 134]. Secondly, there is evidence that contrast causes direct tubular toxicity [133–135]. Regardless of the underlying pathophysiology, the end result is a nephrotoxic phenotype consistent with AKI. Typically, the creatinine increases 24–48 h after contrast administration; resolution of AKI commonly occurs within 5–10 days [133–135]. The AKI is commonly non-oliguric and may be associated with a prerenal fractional excretion of sodium (FENa < 1%) [136].

Early studies suggest that the incidence of contrast-associated AKI was 3–14.5% [135]; incidence is highly associated with underlying risk factors, and some studies suggest the incidence among those with multiple risk factors is 20–30% [137]. However, more recent, well-designed studies of newer agents suggest that in the absence of significant risk factors, the incidence may be much lower than historically thought. The newer data suggesting a lower nephrotoxic risk for contrast has led to a transition away from the concept of contrast-induced nephropathy or AKI to contrast-associated nephropathy or AKI [138]. This sematic change removes the causal concept. In many situations, patients who receive contrast have other AKI risk factors precluding a true causal link to be drawn to the contrast itself. For an episode of AKI to be considered contrast-induced, it must be possible to exclude all other causes of AKI, allowing the causal association to be drawn.

Several risk factors for contrast-associated AKI have been described. First generation iodinated contrast agents were both ionic and hyperosmolar (relative to plasma with osmolarities of >1400 mOsm/kg) [139]. Newer agents include both low-osmolar (500–800 mOsm/kg) and iso-osmolar agents (~300 mOsm/kg); the low-osmolar agents may be ionic or nonionic and the single currently available iso-osmolar agent is nonionic [137]. When compared with the newer low- and iso-osmolar agents, the original hyperosmolar agents are associated with increased nephrotoxic risk [140]; this, in part, may explain the fact that newer studies identify a lower risk of nephrotoxicity with contrast administration. In addition to the type of agent used, the dose administered seems to affect AKI risk with higher doses associated with greater risk [141]. Finally, the type of procedure may impact risk as intra-arterial administration of contrast is associated with greater AKI risk than intravenous administration [133]. Patient-specific risk factors may include administration of additional nephrotoxins, hypovolemia of any cause (dehydration, heart failure, sepsis), albuminuria, diabetes mellitus, and CKD [138].

The relationship between CKD or GFR and contrast-associated nephrotoxicity warrants additional discussion. It is clear that AKI risk is higher in patients with higher CKD stage or lower GFRs [138, 142, 143]. For example, the incidence of contrast-associated AKI has been estimated as ~5% for GFR > 60 mL/min/1.73m^2, 10% for GFR 45–59 mL/min/1.73m^2, 15% for GFR 30–44 mL/min/1.73m^2, and 30% for GFR < 30 mL/min/1.73m^2 [138]. Interestingly, the risk in children may be lower than these rates which were primarily based on adult studies. Two large retrospective studies in children found no increased risk for AKI following intravenous contrast administration regardless of GFR and ambulatory/inpatient setting [144, 145].

Nonsteroidal Anti-inflammatory Drugs

The use of NSAIDs dates back over 3500 years ago to the African continent with the Sumerians using the extracts of willow leaves to treat pain. This extract has been used globally by the Greeks and the Romans in Europe, by the Chinese in Asia, and by Native Americans in North America [146]. The first formal extraction of the active component of the willow tree, salicin, is credited to the Italian pharmacists Fontana and Rigatelli in 1824. Chemical synthesis of acetylsalicylic acid was accomplished in France before the method was patented by a German team in 1874. Twenty-five years later, in a Bayer laboratory, a reliable process for obtaining the purified form was created and the name aspirin was coined [147]. There are now six major classes of NSAIDs with over fifty different formulations and combinations. NHANES III reported that approximately 62% of the US population over the age of 17 took NSAIDs, and it is likely that drugs in the NSAID class are the most ubiquitously administered nephrotoxins worldwide [148].

NSAIDs inhibit the ability of cyclooxygenase (COX) to convert arachidonic acid to prostaglandins and thromboxane. There are two isoforms of COX, COX-1 and COX-2; COX-1 is present in most tissues and is responsible for the basal production of prostaglandin synthesis for homeostatic functions while COX-2 is constitutively expressed primarily in the brain, kidney, ovary, and uterus [149]. Notably, COX-2 is inducible by pro-inflammatory cytokines such as IL-1 and TNF-A. While the primary phenotype of NSAID nephrotoxicity is AKI, disruption of homeostatic physiology can lead to electrolyte disturbances (hyperkalemia or hyponatremia), edema, and papillary necrosis [150–152]. It is likely that the nonspecific nature of COX blockade and the resultant interference with prostaglandin synthesis is primarily responsible for the nephrotoxicity of NSAIDs. The prostaglandins PGE_2 and PGI_2 are responsible for renal vasodilatation, which increases renal blood flow and GFR. Disruption of this vasodilatory process becomes more clinically apparent in scenarios where renal perfusion is already compromised (e.g., heart failure, intravascular volume depletion, sepsis) or in cases where vasoconstricting hormones, such as angiotensin II, are also upregulated (e.g., concomitant administration of ACE inhibitors) [153]; in these cases, NSAID administration may lead to a substantial decrease in GFR and overt AKI. Although the specific COX-2 inhibitors were developed to mitigate the adverse effects seen with nonselective COX inhibition, it turns out that COX-2 production is upregulated in hypovolemia and this enzyme plays an outsized role in the renal protective response. Thus, the risk of AKI with COX-2 inhibitors is similar to that of nonselective inhibitors [154]. While NSAIDs can be used safely when attention is paid to their potential for nephrotoxic risk, AKI occurs in up to 5% of patients receiving these agents [1, 22, 151]; this risk may be exacerbated by any sort of intravascular volume depletion as described above, as well as in the setting of reduced GFR or CKD [44, 94]. In the setting of severe dehydration or massive overdose NSAIDs can even cause acute renal papillary necrosis. The vascular blood supply in the renal papillae is highly dependent on prostaglandin production and impaired production can cause ischemia. Clinically, these patients may present with abdominal or flank pain and gross hematuria. While renal function may return to baseline, maximal concentration of the urine may be impaired due to the loss of the deep loops of Henle [151].

Prostaglandins modulate sodium and water handling and NSAIDs use can lead to water and sodium retention [155]. This effect is particularly pronounced in patients with preexisting positive sodium and water balance such as those in heart failure or with nephrotic syndrome. Although both sodium and fluid are retained, on balance NSAIDs tend to cause hyponatremia, especially in those with generous free water intake [156]. Hyperkalemia is uncommonly seen with NSAID use unless other agents prone to cause potassium retention are administered concomitantly (e.g., potassium-sparing diuretics, potassium supplements, ACE inhibitors, or trimethoprim therapy) [151]. In these cases, inhibition of PGE_2 and PGI_2 reduces the release of renin and aldosterone, further impairing potassium excretion. Hyperkalemic risk may also be exacerbated by higher sodium reabsorption rates as reduced sodium delivery to the distal tubule also decreases effective potassium excretion [155]. Finally, NSAIDs can lead to AIN which may present similarly to AKI. However, patients with AIN are more likely to have systemic symptoms including fever, joint or abdominal pain, and rash [22]. While sterile leukocyturia may be present, this is not a ubiquitous finding and definitive diagnosis requires a kidney biopsy. Distinguishing between AKI and AIN is important as management between the conditions may differ [157].

Renin-Angiotensin-Aldosterone System Inhibitors

The RAAS is primarily responsible for regulating systemic vascular resistance and effective circulating volume. Inhibitors of the RAAS, including ACE inhibitors and ARBs, are commonly prescribed and extraordinarily effective; they are considered first line antihypertensives, they reduce mortality in the setting of heart failure, and slow progression of proteinuria-related kidney disease of diabetic and nondiabetic origin. Despite their effectiveness, they have several side effects including a reduced GFR and are widely considered nephrotoxic by prevailing adult and pediatric nephrology guidelines [36, 71]. When seen, the most common nephrotoxicity phenotype of RAAS blockade is AKI.

The mechanism of AKI in the setting of ACE inhibitor and ARB exposure is hemodynamic alteration of glomerular vasculature as they preferentially inhibit vasodilatation of the afferent arteriole [158]. This effect, when combined with a drop in systemic blood pressures, reduces renal perfusion and GFR, leading to higher serum creatinine levels diagnostic of AKI [159]. Notably this effect is more pronounced in the setting of already reduced effective circulating volume (dehydration, hepatorenal syndrome, sepsis, heart failure, etc.) and the presence of other nephrotoxins (NSAIDs) [160]. For example, in high-risk adults (vascular disease and severe diabetes mellitus), subjective "renal dysfunction" severe enough to require drug discontinuation occurred in 10.2% and 10.6% of patients receiving ramipril (ACE inhibitor) and telmisartan (ARB), respectively [161]. Similarly, in adults undergoing cardiothoracic surgery, pre-operative use of RAAS inhibitors increased the risk for postoperative AKI [162]. In children, a retrospective analysis of 332 patients who received an ACEI found that 20 (6%) experienced a doubling of serum creatinine (KDIGO Stage 2 AKI) [163]; by comparison, the prevalence of AKI was 21% in children receiving ACEI following cardiac surgery [164]. It is important to note that this hemodynamic effect is typically reversible with drug cessation and there is some debate as to the long-term renal risk associated with administration of these medications [165]; some have even advocated for maintaining these medications in the setting of early AKI [158, 159]. As such, it is important to balance the long-term cardiovascular and renal benefits afforded by these medications with the potential for acute episodes of nephrotoxicity. However, the effect on renal perfusion is clear and with the increasing complexity seen in pediatric disease management, it is important to think of these medications as capable of causing or exacerbating nephrotoxic AKI, especially in the setting of substantial nephrotoxin burden.

Prevention and Management

There is no pharmacologic therapy capable of treating or curing nephrotoxic AKI once it develops. However, delayed recognition and intervention may result in disease progression and irreversible damage. Thus, the cornerstones of management are prevention, risk minimization, early identification, and severity mitigation (Fig. 2). The pathophysiologic principles described previously in this chapter form the foundation of management strategies and a thorough understanding of the manner in which substances cause injury will help guide practitioners as they navigate the complex interplay between a patient's condition, underlying comorbidities and disease related risk factors, and polypharmaceutical nephrotoxic burdens. Furthermore, a more detailed understanding of the most commonly prescribed agents, as highlighted in the previous section, will allow for optimization of therapeutic approach even in the most nuanced clinical scenarios.

If, indeed, the mechanism of injury is known, interventions capable of mitigating damage may exist. For example, if NSAID-associated AKI fails to improve following discontinuation of the medication, a renal biopsy looking for AIN may be indicated. Although their effectiveness is somewhat debated, if AIN is confirmed, corticosteroids may be beneficial [95, 166–168]. Acyclovir provides another excellent example of this concept. As the mechanism of injury is urinary crystal formation, volume expansion and/or generation of brisk urine flow may help prevent crystal precipitation and tubular obstruction or, at a minimum, reduce its severity [169]. Notably, while diuretics have been used to augment urine output, they really should only be used to manage volume overload (if it occurs) as forced diuresis without adequate volume expansion may cause hypovolemia, decrease renal perfusion, and worsen renal injury [42]. Additionally, some nephrotoxic medications such as methotrexate

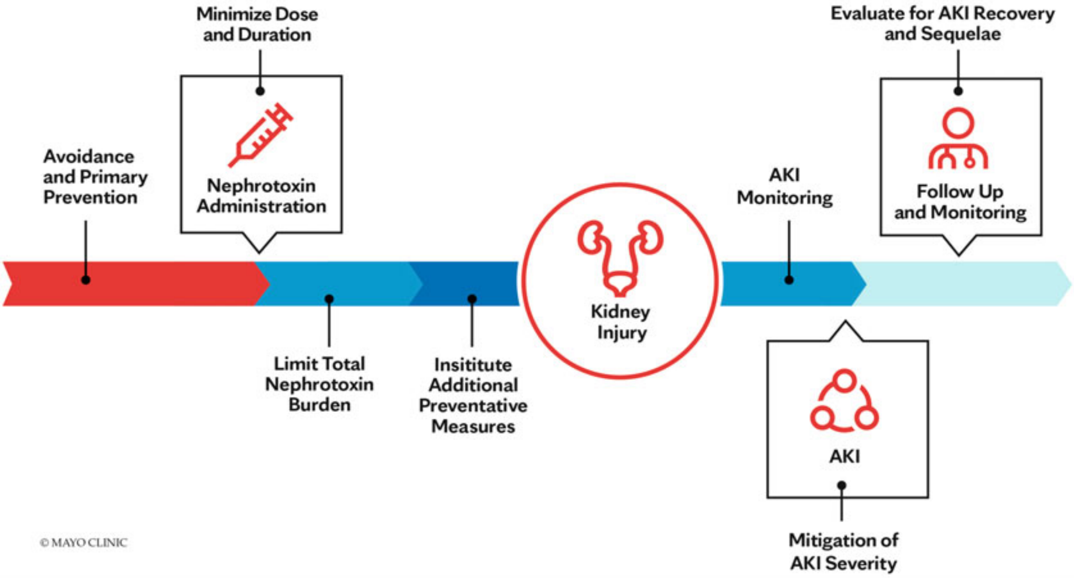
Minimize Dose and Duration
Evaluate for AKI Recovery and Sequelae
Avoidance and Primary Prevention
Nephrotoxin Administration
AKI Monitoring
Follow Up and Monitoring
Kidney Injury
Limit Total Nephrotoxin Burden
Insititute Additional Preventative Measures
AKI
Mitigation of AKI Severity
© MAYO CLINIC

Fig. 2 Approaches to the management of nephrotoxins and nephrotoxic AKI. Although specific treatments do not exist for nephrotoxic injury once it develops, successful strategies have been developed to guide the care of patients requiring administratoin of these substances. The earliest possible intervention is avoidance (primary prevention). If the risk of the medication outweighs the benefit, it should not be administered; likewise, if a less injurious but equally effective alternative is available, substitution should be considered. Secondly, if nephrotoxins are indeed required, it is of paramount importance to minimize the dose and durtation of individual medications as well as to limit the overall, comprehensive nephrotoxin burden. Third, if specific preventative strategies are available (e.g., volume resusitation with acyclovir administration), they should be proactively deployed. Should injury occur, it is important to identify the presence (diagnose) and severity (stage) of AKI as well as to monitor for disease progression. Once AKI develops, all other potential AKI risk factors should be evaluated and eliminated or modified to the extent possible. Finally, among all patients who develop injury, it is imperative that these patients receive appropriate follow-up care for their adverse event; this includes assessing AKI recovery and evaluating for long-term sequelae (e.g., hypertension, proteinuria, CKD). AKI acute kidney injury. (Used with permission of Mayo Foundation for Medical Education and Research, all rights reserved)

and sulfadiazine are known to have higher solubility in an alkaline urine (pH >7); thus, administration of bicarbonate containing intravenous fluids may prevent crystal formation and support dissolution of existing crystals. However, caution must be exercised as serum electrolytes shifts and urinary calcium phosphate precipitation are potential consequences of alkaline fluid administration; it is imperative that the treatment is no worse than the disease [170]. It is important to note that a solid understanding of pathophysiology is not always sufficient, and some diseases continue to wait for effective treatments. Contrast nephropathy is a great example. Although the manner in which injury occurs is fairly well understood, attempts to prevent contrast-associated AKI with n-acetylcysteine and other antioxidants have proven fruitless [171, 172]. Some agents, such as the adenosine receptor antagonists (theophylline and aminophylline) have shown an effectiveness in observational studies that has not always been confirmed in controlled trials [173–175]. Despite many setbacks, interest remains high in developing therapeutics with the goal of preventing or treating AKI of all causes. Not only will the pathophysiology of nephrotoxic AKI guide the development of targeted therapeutics, but it will also allow providers to more efficiently cross-purpose agents found to be effective in other types of AKI.

As definitive treatment for AKI remains elusive; early and accurate AKI detection would provide time to optimize care and mitigate injury. With the adoption of electronic medical records

(EMR), there is tremendous potential although utilizing the large of amount of data in the EMR to effect positive outcomes has not proven to be simple [176]. While a comprehensive discussion of EMR alerts and interventions is beyond the scope of this chapter, it is clear that broad and timely awareness of nephrotoxin exposure and injury is a necessary first step, effective detection systems can be developed, and that the optimal approach is highly dependent on the desired outcome, resource availability, and institutional willingness to accept change management. The use of electronic alerts has been effective when the target of the alert has been front-line providers or an imbedded pharmacist; a positive impact on process outcomes (e.g., reduction of nephrotoxin exposure) has been reported in both smaller pediatric and larger adult studies [177, 178]. It has been more challenging to assess the impact on higher-level patient-related outcomes such as mortality and the need for renal replacement therapy [179–181]. It is clear that highly relevant care recommendations (also referred to as clinical decision support) must accompany any nephrotoxin or AKI-related alert if the notification is to be impactful [182]. Even better than identifying nephrotoxin exposure and nephrotoxic AKI once it develops, is the ability to actually predict exposure and injury. Interest in AKI prediction models has grown substantially over the past decade and many of the earliest models focused specifically on reduction of nephrotoxin-associated AKI [183]. Operational challenges abound but EHR enabled prediction of nephrotoxic AKI remains one of the most promising interventional approaches in theory [176, 184, 185].

One model that has been particularly successful at affecting nephrotoxic AKI is NINJA. Originally a single center program, NINJA was expanded to a multicenter collaborative, which has achieved a reduction in nephrotoxic AKI among hospitalized children receiving noncritical care of nearly 25% [36]. The NINJA model is built on the concept that AKI risk increases with concomitant nephrotoxin exposure and that awareness of this risk has the capacity to modify care and outcomes [186]. NINJA identifies patients receiving a high burden of nephrotoxins and uses this exposure to trigger daily serum creatinine monitoring. The ordering of serum creatinine is multifaceted; it increases awareness of risk, it establishes a protocol to monitor for the adverse event associated with exposure (AKI), and it provides the basis for an injury alert should it occur. Decision support is provided for the duration of exposure, most typically by pharmacists who can recommend alternative therapies. Given its success in noncritically ill children, NINJA has been successfully applied in the neonatal intensive care unit as well [187]. Most recently, the NINJA model was adopted as a pilot program with the safety collaborative Solutions for Patient Safety (SPS), a North American network of >140 pediatric centers. This pilot will attempt to assess the impact of the NINJA bundle across a wide variety of institutions and patient populations.

The future of prediction modeling for nephrotoxin-associated AKI may include a role for renal biomarkers. Again, a complete discussion of biomarkers is beyond the scope of this chapter, but it is likely they will become an integral part of prevention and management strategies in the future. Cellular damage markers such as neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-β-D-glucosaminidase (NAG), tissue inhibitor of metalloproteinase 2 insulin-like growth factor-binding protein 7 ([TIMP-2]·[IBFBP7]) and kidney injury molecule-1 (KIM-1) have been evaluated in the setting of nephrotoxin exposure [188]. Additional studies are still needed to validate early findings; however, preliminary data are promising [189]. It is likely that biomarkers will be used in conjunction with clinical risk scoring systems or existing clinical interventions such as NINJA to optimize predictive capabilities and effectively reduce nephrotoxic injury [190–192].

Conclusion

In summary, a myriad of nephrotoxin substances exist and while the primary focus in the literature has been on pharmaceutical agents, it is important to remember that naturally occurring compounds and environmenal exposures can cause kidney injury as well. Through various pathophysiologic mechanisms, nephrotoxins may cause glomerular disease, crysalluria, and disruptions of fluid/electrolyte homeostasis; however, the most commonly seen phenotype is AKI. While it is important to understand the nephrotoxic potential of any given agent, it is possible that the concept of a patient's comprehensive nephrotoxin burden is of greater clinical relevance. An understanding of the core concepts that underpin nephrotoxicity allows provider to effectivley manage both exposures and nephrotoxic injury. Although specific intervetions for nephrotoxic AKI do not yet exist, multidisciplinary approaches designed to increase awareness, reduce exposure, and indentify injury have proven quite effective. Over time, novel approaches to identify, prevent, and reverse injury will hopefully reshape our approach to nephrotoxins and nephrotoxic AKI.

Cross-References

- ▶ Assessment of Kidney Function in Children, Adolescents, and Young Adults
- ▶ Evaluation and Management of Acute Kidney Injury in Children
- ▶ Pathogenesis of Acute Kidney Injury
- ▶ Pediatric Fanconi Syndrome
- ▶ Pediatric Tubulointerstitial Nephritis
- ▶ Technical Aspects of Renal Pathology

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Part XI

Chronic Kidney Failure

Progression of Chronic Kidney Disease and Nephroprotection in Children

69

Elke Wühl and H. William Schnaper

In memory of H. William Schnaper, an outstanding person and a great teacher who paved the way to understanding the pathophysiology of chronic kidney disease

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Abstract

Progression of chronic kidney disease (CKD) towards kidney replacement therapy is inevitable in some CKD patients and once significant impairment of kidney function has occurred it tends to progress almost irrespectively of the underlying kidney disorder.

In addition to the key players hypertension, proteinuria, and the renin-angiotensin system, other potential contributors such as the underlying kidney disease, age at onset, gender, genetic background, metabolic acidosis, renal anemia, altered mineral homeostasis, dyslipidemia, hyperuricemia, inflammation, and oxidative stress as well as general cardiovascular risk factors such as diabetes, smoking, and obesity also play a role. The current state of knowledge regarding the pathophysiology of kidney disease progression and therapeutic concepts will be discussed in this chapter.

Keywords

Progression · Chronic kidney disease · Renoprotection · Hypertension · Proteinuria · Children · Adolescents

Introduction

Progression of chronic kidney disease (CKD) towards kidney replacement therapy (KRT) is inevitable in some CKD patients and once significant impairment of kidney function has occurred it tends to progress almost irrespectively of the underlying kidney disorder. While the primary disease-causing renal impairment vary, as CKD progresses the mechanisms involved become increasingly similar and ultimately a set of common events contributes to an unstoppable loss of functional nephron mass.

There is clear evidence from clinical studies that both hypertension and proteinuria are key players in the pathophysiology of CKD progression in humans [1–3]. In addition to the renin-angiotensin system other potential contributors, such as the underlying kidney disease, age at onset, gender, genetic background, metabolic acidosis, renal anemia, altered mineral homeostasis, dyslipidemia, hyperuricemia, inflammation, and oxidative stress as well as general cardiovascular risk factors such as diabetes, smoking, and obesity, also play a role.

This chapter summarizes the current state of knowledge regarding pathophysiology of kidney disease progression and therapeutic concepts.

Pattern and Timing of CKD Progression

Several studies have described the pattern of CKD progression in glomerular and non-glomerular pediatric kidney disease.

Data from the prospective, population-based ItalKid Project [4] showed a yearly incidence of KRT of 7.3% with a 68% cumulative risk of KRT by age 20 in children and adolescents with CKD registered in Italy between 1990 and 2000. The loss of renal function was not linear, but was characterized by a greater decline around puberty. The probability of renal survival decreased with lower renal function at baseline.

In a retrospective analysis of 176 patients with renal hypodysplasia from Great Ormond Street Hospital, London, it was suggested that the natural course of kidney function in patients with congenitally reduced kidney mass can be divided into three periods: an initial “hypertrophy” phase characterized by improving kidney function during the first 3 years of life, and a subsequent period of stable kidney function attained by 50% of the patients and lasting for a mean of 8 years, followed by a period of gradually deteriorating kidney function towards KRT [5]. The progression phase started just after infancy in 48% and around puberty in 23% of patients. In 30% kidney function remained stable even beyond puberty. Correlates of deteriorating function were proteinuria, hypertension, urinary tract infections, and lower glomerular filtration rate (GFR) at onset.

In a recent analysis of 4,700 patients with congenital anomalies of the kidneys and the urinary tract (CAKUT) followed in the ERA-EDTA Registry, more than two-thirds of the patients required KRT in adult age, with a mean age at starting KRT of 34 years [6]. These findings support the concept that in patients with a congenital reduction in nephron mass, CKD progression due to ongoing loss of remnant nephrons is a lifelong process.

In a joint analysis of more than 1200 children with glomerular and non-glomerular CKD followed in the CKiD (Chronic Kidney Disease in Children) study and the ESCAPE (Effect of

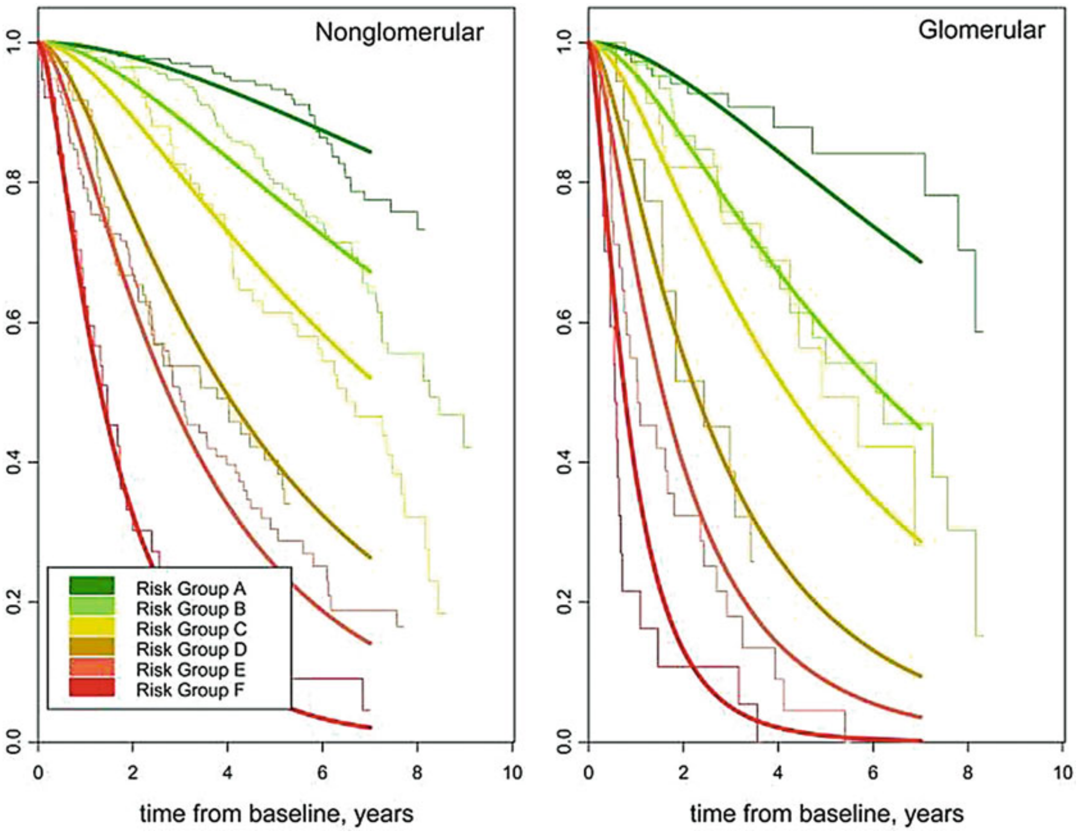

Fig. 1 Survival curves for the time from assessment of CKD (baseline) to a composite clinical event (50% GFR decline, kidney replacement therapy, or GFR less than 15 ml/min/1.73 m^2) stratified by CKD diagnosis and risk group A to F. Risk Group: **A** – CKD stage I to IIIa and UPCR < 0.5; **B** – CKD stage IIIb and UPCR < 0.5 *or* CKD stage II to IIIa and UPCR 0.5 to 2; **C** – CKD stage IIIb and UPCR 0.5 to 2 *or* CKD stage II and UPCR > 2; **D** – CKD stage IV and UPCR < 0.5 *or* CKD stage IIIa and UPCR > 2; **E** – CKD stage IV and UPCR 0.5 to 2 *or* CKD stage IIIb and UPCR > 2; **F** – CKD stage IV and UPCR > 2. (This figure was published in [7], Copyright Elsevier, with permission)

Strict Blood Pressure Control and ACE Inhibition on Progression of Chronic Renal Failure in Pediatric Patients) trial, children with glomerular disease were estimated to have a 43% shorter time to a composite event of KRT or a 50% GFR reduction or GFR <15 ml/min/1.73 m^2 than children with non-glomerular disease [7]. By staging CKD according to level of GFR and proteinuria, timelines of progression were defined that may guide management strategies in children with CKD. While in children with mild-to-moderate CKD without proteinuria the median time to attainment of the composite endpoint was longer than 10 years, in children within the highest risk group, defined by CKD stage IV and gross proteinuria, 50% of children with non-glomerular CKD reached the composite endpoint within 1.3 years and with glomerular CKD within 0.8 years (Fig. 1).

Renal Physiology of Progressive CKD

Progression of kidney disease can be considered to occur in three phases: First, events related to the initial disease, involving a harmful stimulus and tissue response. Second, conversion from an acute disease state to chronicity and third, a period of progressive decline leading to end-stage kidney disease (ESKD). While in adults the most common causes for CKD are diabetes and hypertension [8], in children the initial insult to the kidney is of a more diverse origin, including CAKUT with hypodysplasia or cystic kidney diseases, metabolic kidney diseases, congenital nephrotic syndrome or acquired glomerulopathies, and hemolytic-uremic syndrome [9].

Although each disorder gives a different pattern of injury, common mechanisms come into play in response to injury. The response of the kidney to a precipitating injury is a critical determinant of outcome. Repair after injury, via both general cellular mechanisms and responses specific to the kidney, may lead to healing or to malfunction and kidney scarring. Basically, the involved repair mechanisms are normal and appropriate attempts to restore nephron function, however, when structural problems or inappropriately balanced responses occur, a state of *misdirected repair* leads to an increase in the loss of function. Physiological responses to the repaired state lead to *adaptation* by the nephron and may result in a new, effective steady state, while excessive responses may lead to abnormal physiology and further cycles of nephron loss and adaptation. Because this leads to long-term deleterious effects in the nephron, such negative responses might be referred to as *maladaptation*.

Primary glomerular injury. Genetic and possibly environmental stimuli lead to podocyte injury resulting in failure of the glomerular filter and proteinuria.

Loss of podocytes permits activation of mesangial and endothelial cells in the glomerular tuft [10]. Likewise, other cell types may contribute to glomerular disease. The initial mesangial location of extracellular matrix (ECM) accumulation in many glomerular diseases suggests that mesangial cells are a primary source of scar tissue [11]. Mesangial cells may also undergo proliferation after a number of stimuli; however, it is not clear whether these mesangial cell responses represent a primary manifestation of injury or a result of misdirected repair. Also, glomerular endothelial cells may proliferate in response to hypertrophic stimuli undergoing phenotypic changes that contribute to scarring [12].

The loss of damaged podocytes may lead to focal and segmental adhesions of the denuded basement membrane of the Bowman capsule, with spreading of the ultrafiltrate into the tubulointerstitial compartment where it causes inflammatory injury (“misdirected filtration”). The remaining nephrons lose their ability to autoregulate glomerular pressure, resulting in transmission of systemic hypertension to the glomerulus. Increased intraglomerular pressure induces proteinuria, which is the pathophysiological link between glomerular, interstitial, and tubular damage.

Tubular injury. Tubular injury and dysfunction may occur secondary to chronic glomerular disease but may also be primary. Chronic tubulointerstitial injury early in life often results from genetic disorders that cause structural disorientation (genetically inherited cystic kidney disease or cystic dysplastic kidney), organellular accumulation of materials that inhibit normal cellular function (cystinosis or Lowe syndrome), or metabolic failure. Other potential causes are obstruction, infections, and toxins, as well as hypoxic/ischemic insults. A common event in the progression of all of these disorders is the occurrence of tubulointerstitial scarring and inflammation, associated loss of nephrons, and their replacement with scar tissue [13].

Tubulointerstitial injury. Renal tubular delivery of biologically active molecules may lead to inflammation and injury to the tubular cells or the tubulointerstitium. In addition, increased distal delivery of filtrate activates the renin-angiotensin-aldosterone system (RAAS) to modify glomerular filtration. This tubulo-glomerular feedback may provide the hypertrophic stimulus described above. In both glomerular and tubulointerstitial disease, cell activation, dedifferentiation, and redifferentiation to a cellular phenotype that mediates attempted repair are stimulated. However, failure of that repair leads to scarring involving *inflammation* and *fibrogenesis,* as is the case in CKD progression.

Inflammation. Progression of focal segmental glomerulosclerosis (FSGS) to ESKD is most strongly associated with inflammatory infiltrates in the tubulointerstitium, suggesting an important role of inflammation in CKD progression.

Macrophages, found both in glomeruli and the tubulointerstitium, exert not only phagocytic function but also synthetic function which is a critical mediator of both repair and scarring. Regulatory cytokines produced by the macrophage include transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), basic fibroblast growth factor (FGF-2), and tumor necrosis factor (TNF)-α. Macrophages also produce regulators of coagulation and fibrogenesis, complement-related proteins, endothelin-1, and reactive oxygen species. A major determinant of macrophage recruitment is regulatory molecules governing cell adhesion and migration. Injury also activates various other cells in the kidney to express specific chemokines and their receptors playing a role in fibrosis.

Fibrogenesis. In normal tissue, ECM synthesis and degradation are balanced by components regulating ECM protein expression, degradation, and proteolytic enzyme activity. Synthesis of the ECM proteins is regulated at the level of transcription, stimulated by mediators such as TGF-β, and by enzymes that process the ECM proteins both intracellularly and extracellularly to promote ECM incorporation into organized matrices. The maintenance of appropriate balance among the ECM proteins involves both the extracellular matrix proteases such as matrix metalloproteinase (MMP)-2 and urokinase-type plasminogen activator (uPA), and their naturally occurring inhibitors tissue inhibitor of metalloproteinase (TIMP)-2 and plasminogen activator inhibitor (PAI)-1, respectively.

The myofibroblast is the major cellular contributor to fibrogenesis by very high synthetic capacity and production of large amounts of ECM. It has been hypothesized that myofibroblasts are derived from other cells in the kidney by the process of epithelial-to-mesenchymal transition (EMT). However, even kidney cells that do not undergo EMT may still contribute to progression by producing chemoattractants, procoagulants, and profibrotic factors. In addition, delivery of large amounts of plasma to the tubular lumen increases exposure of the luminal side of the tubule to cytokines and growth factors, which could also stimulate cells to undergo EMT and produce additional mediators.

Role of the renin-angiotensin-aldosterone system (RAAS) in CKD progression. Angiotensin II (Ang II)*,* the primary effector of the RAAS, is mechanistically involved in most of the processes described above. Renin release from the juxtaglomerular cells drives systemic Ang II generation. Most of the intrarenal effects of Ang II are mediated via the angiotensin (AT)1 receptor. Ang II is a potent vasoconstrictor that augments intraglomerular pressure by increasing the efferent arteriolar tone. Furthermore, Ang II increases intracellular calcium in podocytes inducing cytoskeletal changes and altered podocyte function with induction of protein ultrafiltration, the proliferation of smooth muscle cells, and the glomerular and tubular expression of various growth factors, cytokines, and chemokines. Ang II also stimulates *oxidative stress*, which perpetuates the upregulation of cytokines, adhesion molecules, and chemo-attractants. Finally, intrarenal Ang II stimulates neuronal afferences which are assumed to activate central nervous structures regulating the sympathetic tone. Hence, Ang II is pathophysiologically involved in the state of sympathetic hyperactivation that is characteristic for CKD and represents another important mechanism for renal disease progression and cardiovascular morbidity.

In patients with reduced nephron mass and anemia, hypoxia of tubular cells is additionally favored by an increased oxygen consumption by tubular cells of the remaining nephrons, a decreased number of interstitial capillaries and accumulation of extracellular matrix. Conversely, hypoxia stimulates fibrosis and enhances the local production of reactive oxygen species. Oxidative stress also induces the release of pro-inflammatory and pro-fibrotic molecules, thereby enhancing the production of extracellular matrix by fibroblasts favoring tubular cell death (Fig. 2).

Nephron hypertrophy. With a decrease in renal mass, renal blood flow perfuses fewer nephrons with consequently increased single-nephron plasma flow and GFR. Hyperperfusion and hyperfiltration may stretch the nephron, which can induce hypertrophic and hyperplastic signaling with increased activity of the RAAS.

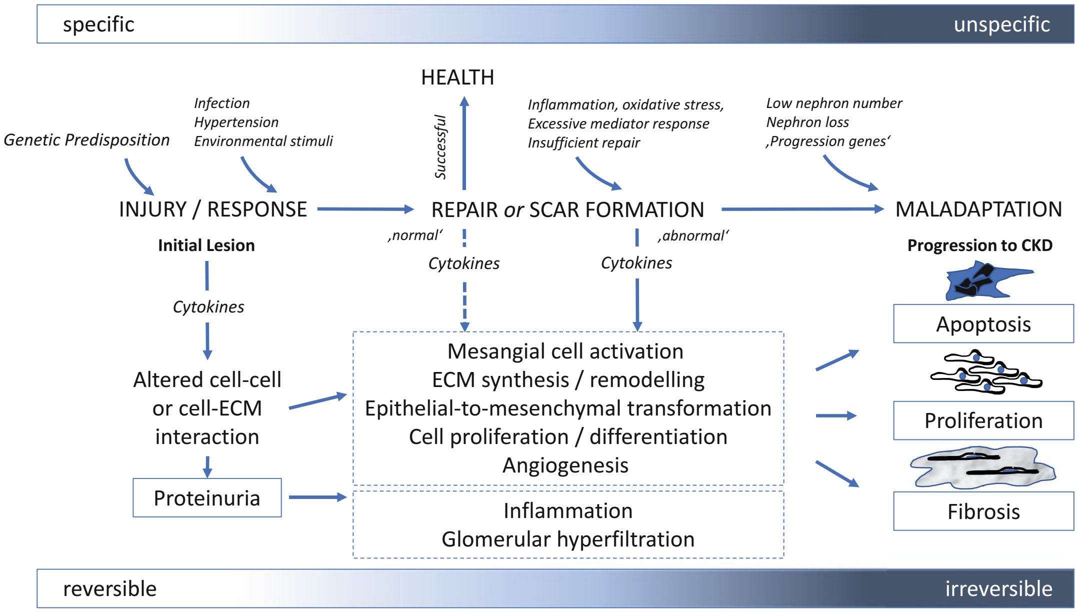
specific
unspecific
HEALTH
Successful
Genetic Predisposition
Infection
Hypertension
Environmental stimuli
Inflammation, oxidative stress,
Excessive mediator response
Insufficient repair
Low nephron number
Nephron loss
‚Progression genes'
INJURY / RESPONSE
REPAIR or SCAR FORMATION
MALADAPTATION
Initial Lesion
‚normal'
‚abnormal'
Cytokines
Cytokines
Progression to CKD
Cytokines
Altered cell-cell
or cell-ECM
interaction
Proteinuria
Mesangial cell activation
ECM synthesis / remodelling
Epithelial-to-mesenchymal transformation
Cell proliferation / differentiation
Angiogenesis
Inflammation
Glomerular hyperfiltration
Apoptosis
Proliferation
Fibrosis
reversible
irreversible

Fig. 2 Clinical and pathogenic stages from initial injury to progressive kidney disease. As chronicity increases, pathogenic mechanisms become increasingly similar to those in other conditions. *ECM* extracellular matrix, *CKD* chronic kidney disease

Chronic nephron loss. Glomerular sclerosis, tubulointerstitial fibrosis, and tubular atrophy result in a further loss of functioning renal mass, closing a vicious circle by further increasing intraglomerular pressure and hypertrophy of the remaining glomeruli.

Risk Factors for CKD Progression

Nonmodifiable Risk Factors

Underlying Kidney Disease and Genetic Disposition

A major determinant of rates and patterns of progression is the primary etiology of the underlying kidney disease [14]. The most common causes of CKD and KRT in children are diseases related to CAKUT [4, 14, 15], followed by glomerular disease, neurogenic bladder, and other disorders. In children, hypodysplasia accounts for 57.5% of CKD patients, but only 39.6% of those with KRT [4], while patients with underlying chronic glomerulonephritis or FSGS comprise 4.4% of CKD but 10.7% of KRT patients. These results suggest that patients with glomerular lesions progress more rapidly to KRT [6, 16].

The progression phenotype may be linked to an *underlying genetic defect*. In disease entities caused by defects in more than one gene, progression patterns may differ according to the gene involved. Even within the same gene, the localization and type of the causative mutations may be a key determinant of the renal prognosis.

Additionally, common *polymorphic variants* in various genes encoding cytokines, growth factors, and regulatory peptides may determine an individual's susceptibility for renal injury, renal disease progression, and the response to renoprotective treatment.

Recent and ongoing meta-analyses of genome-wide association studies (GWAS) are aiming to identify loci associated with incident CKD or the risk of ESKD in general population-based cohorts, as well as in adult and pediatric CKD cohorts. For instance, common risk variants of genes related to nephrogenesis (*ALMS1, VEGFA,* and *DACH*), in glomerular filtration barrier formation and podocyte function (*DAB2, PARD3B,* and *VEGFA*), angiogenesis

(*VEGFA*), and to renal function (*UMOD, SHROOM3,* and *STC1*) have been identified [17–19]. Genome-wide association studies may also allow identification of variants related to known risk factors for CKD progression as hypertension, proteinuria, or anemia in adults as well as children [20].

Age at Attainment of Renal Mass Deficit

The age at which a significant loss of kidney mass occurs may influence the degree of glomerular hypertrophy and the long-term prognosis for kidney survival. Patients born with unilateral renal agenesis or multicystic dysplastic kidney are at greater risk for proteinuria, hypertension, and loss of kidney function than subjects after loss of one kidney in later life due to an accident or after kidney donation, suggesting that the number of functional nephrons in congenital solitary kidneys might be decreased [21].

Fetal Programming

Fetal programming describes a process by which changes to the intrauterine environment increase the susceptibility of a fetus to diseases later in life. Fetal programming induces epigenetic changes that are modified postnatally and may have direct consequences on kidney development and function. It has been shown by Barker and colleagues that low birth weight is correlated with the incidence of cardiovascular disease in later adulthood [22]. Subsequent studies have suggested that low nephron number predisposes to adult hypertension and there is a clear correlation of low birth weight with decreased kidney mass and nephron number [23]. A recent study in more than 400 Japanese children born with very low birth weight showed a significant contribution of intrauterine malnutrition to chronic kidney damage [24]. Vice versa, higher estimated weights in utero at birth and at 6 months postgestational age are associated with higher kidney volume and higher GFR at age 6–7 years [25]. Although an association between low nephron number and subsequent hypertension or kidney dysfunction is difficult to demonstrate directly, clinical evidence at least suggests such an association.

Growth

In some kidney diseases, the likelihood of CKD progressing to kidney failure is greatest in the first year of life or around the adolescent growth spurt [5], i.e., periods of accelerated overall growth. This led to the hypothesis that somatic growth in the absence of equivalent kidney growth may be a cause of CKD progression due to a relative decrease in the available nephron mass related to body weight. This may again implicate low nephron number as a key risk factor in CKD progression.

Major Modifiable Risk Factors in CKD

Hypertension

Hypertension is not only a well-known cause of kidney failure and a marker of the severity of the underlying kidney disease, but also an independent risk factor for kidney disease progression in adults [1–3].

Hypertension prevalence estimates in children with CKD range from 20% to 80%, depending on the degree of kidney dysfunction and underlying kidney disease. However, even children with CKD stage 2 or renal hypodysplasia, conditions usually not strongly associated with hypertension, may present with elevated blood pressure (BP) [26]. A systolic BP greater than 120 mmHg has been associated with a faster GFR decline in a large prospective study of children with CKD [27]. In a retrospective cohort study of more than 40,000 adult patients included in a hypertension disease registry, systolic BP was associated with incident CKD. Above a systolic BP level of 120 mmHg, each 10 mmHg increase in baseline and time-varying systolic BP was associated with a 6% increase in the risk of developing CKD. Every 10 mmHg increase in systolic BP was associated with an additional 0.2 ml/min/1.73 m^2 decline in eGFR per year [28].

Among CKD children included in the CKiD study, time-varying BP measurements $\geq$ 90th percentile compared to $<$ 50th percentile were associated with a hazard odds ratio of 3.75 in non-glomerular and of 5.96 in glomerular disease of attaining the composite end point of 50% GFR reduction from baseline, or GFR <15 ml/min/1.73m^2, or dialysis or transplant [29].

Independent of the absolute BP level, alterations of the physiological circadian BP variation with attenuation of the nocturnal fall of BP ("dipping" pattern) are associated with an increased cardiovascular risk and a more rapid progression of kidney failure in adult CKD patients [30]. With respect to the comparative importance of absolute nighttime BP values over nondipping status on the prognosis of renal outcomes, nighttime BP demonstrated a better association with both renal outcomes and markers of kidney dysfunction. However, increased nighttime BP and nondipping status usually coexist, and consequently their relative significance might be synergistic, additive, or even indifferent in diverse clinical settings [31].

Proteinuria

Population-based studies in healthy individuals have demonstrated that proteinuria is an independent risk factor for ESKD and overall mortality [32, 33]. In adults with diabetic and nondiabetic kidney disorders, proteinuria is clearly predictive of the renal prognosis [34, 35]. In the Ramipril Efficacy in Nephropathy (REIN) trial [36], urinary protein excretion was the only baseline variable correlated with GFR decline and progression to ESKD. Although the spectrum of underlying kidney disorders in children markedly differs from adults, the "European Study Group for Nutritional Treatment of Chronic Renal Failure in Childhood" first demonstrated in 200 children with CKD stage 3–4 that even in pediatric kidney disease, proteinuria and hypertension are major predictors of GFR decline [27]. The finding that proteinuria predicts kidney disease progression has been confirmed in children with hypodysplastic kidneys by the ITALKID Project [37]. In the CKiD study, a decrease in GFR was associated with an increase in proteinuria [38], and baseline proteinuria and systolic BP levels were independently associated with CKD progression in children with non-glomerular CKD [39]. In the ESCAPE trial, baseline proteinuria and residual proteinuria during ACE inhibition were quantitatively associated with renal failure progression [40, 41].

Additional Contributing Factor

Among children with moderate CKD, ***dyslipidemia*** is common and associated with lower GFR, nephrotic range proteinuria, and non-renal factors including age and obesity [42]. Epidemiological studies give some evidence that dyslipidemia is an independent risk factor not only for cardiovascular disease (CVD) but also for progressive chronic renal failure [43]. The dyslipidemic pattern differs between the major renal disease entities [44] and the degree of dyslipidemia parallels the degree of renal function impairment. Underlying mechanisms of uremic dyslipidemia include insulin resistance [45], obesity, hyperparathyroidism [46], malnutrition, acidosis [47], and impaired catabolism of triglyceride-rich lipoproteins by decreased activity of lipoprotein lipase and hepatic triglyceride lipase.

In addition, there are observations that the insulin resistance syndrome may underlie or mediate the association between lipids and loss of renal function. In humans, a strong relationship between the ***metabolic syndrome*** and the risk for CKD and microalbuminuria was found in a large nondiabetic general population [48].

As adipose tissue generates high levels of circulating free fatty acids (FFA) and FFA uptake and oxidation in CKD are reduced by hypoadiponectinemia, leptin resistance, and increased cytokine release from adipose tissue and macrophages, intracellular FFA accumulates [49]. This may cause mesangial and epithelial cell injury and renal disease progression as a consequence of FFA-induced lipotoxicity. However, the evidence for dyslipidemia as an independent risk factor for renal disease development and/or progression is not as strong in clinical studies as it is in experimental settings.

In adults, ***obesity*** predisposes to diabetic nephropathy and appears to be a negative prognostic factor also in other nephropathies and an independent risk factor for accelerated progression [50]. These effects could relate to the relative ratio of body mass to nephron mass, since weight loss intervention reduces hyperfiltration and proteinuria, but also could relate to other effects on BP and metabolism. A meta-analysis indicated that obesity poses a significant risk for CKD or its exacerbation, both independently and in interaction with other factors. The severity of the risk increases with increasing BMI [51].

In CKD, impaired excretion of ammonia, reduced tubular bicarbonate reabsorption, and insufficient renal bicarbonate production in relation to the amount of acids synthesized by the body and ingested with food lead to acidification and may contribute to an activation of the complement pathway that contributes to inflammation and progressive nephron loss. Studies in adult CKD patients suggest that ***metabolic acidosis*** is associated with faster decline in eGFR [52]. In the Cardiovascular Comorbidity in Children with CKD Study (4C Study), pediatric patients with CKD stage 3–5 with time-varying serum bicarbonate below 18 mmol/l had a significantly higher risk of CKD progression compared to those with a serum bicarbonate of 22 mmol/l or more [53].

Serum uric acid is often elevated in CKD subjects due to decreased renal clearance. Serum uric acid is an independent risk factor for kidney disease in healthy adults, with high levels predictive of decline in GFR. Uric acid levels tend to be higher in obese patients, and obesity is a risk factor for CKD as well [54]. Because impaired renal function may decrease the ability to excrete uric acid, it is difficult to know whether the association of ***hyperuricemia*** with more rapid progression is causal. A single, high uric acid determination was not predictive of kidney failure in the Modification of Diet in Renal Disease (MDRD) study [55]. However, data from the CKiD study indicated that hyperuricemia may be an independent risk factor for faster progression of CKD in children and adolescents [56].

In patients at advanced stages of CKD, increased ***oxidative stress*** is associated with additional complications such as hypertension, atherosclerosis, inflammation, and anemia. Because oxidative stress is implicated in the CKD pathophysiology, as the kidney function deteriorates, the redox status imbalance becomes more profound [57].

Disturbances of the ***bone mineral metabolism*** also may influence CKD progression. Elevated serum alkaline phosphatase and low 25-hydroxyvitamin D levels are risk factors associated with progression. Phosphorus accumulation is caused by loss of nephron mass, but is also an independent risk factor for progression in adults and children [15]. These observations may be related to the role of phosphate accumulation in stimulating fibroblast growth factor 23 (FGF23) [58], which has been identified as an independent risk factor for progression in adults and children. Because CKD-associated mineral and bone disease is a result of decreased renal function, it is difficult to distinguish between cause and effect in progressive loss of renal function. It is likely that both are present; loss of function stimulates FGF23 expression and activity, which induces further progression mechanisms.

In CKD patients, potentially harmful effects of the degree of exposure to ***smoking*** on the progression of CKD have been shown. In the KoreaN Cohort Study for Outcomes in Patients with Chronic Kidney Disease (KNOW-CKD study), smoking was associated with a significantly increased risk of renal disease progression in smokers compared with never-smokers [59]. Similar results were found for children exposed to secondhand smoking. In the CKiD cohort, exposure to secondhand smoke was independently associated with nephrotic range proteinuria, and may be an important factor to consider in CKD progression [60].

The most relevant risk factors for CKD progression are summarized in Fig. 3.

Nephroprotective Treatment Strategies

Based on the current understanding of the mechanisms of kidney disease progression, several nephroprotective strategies have emerged in recent years, mainly based on clinical evidence established in adult patients, but a growing body of evidence supports their efficacy in children too. Approaches to treatment could involve some combination of treating the underlying disease, controlling systemic and intrarenal BP and proteinuria, regulating the fibrogenic and inflammatory response, and addressing the metabolic consequences of progressive kidney disease (Fig. 4).

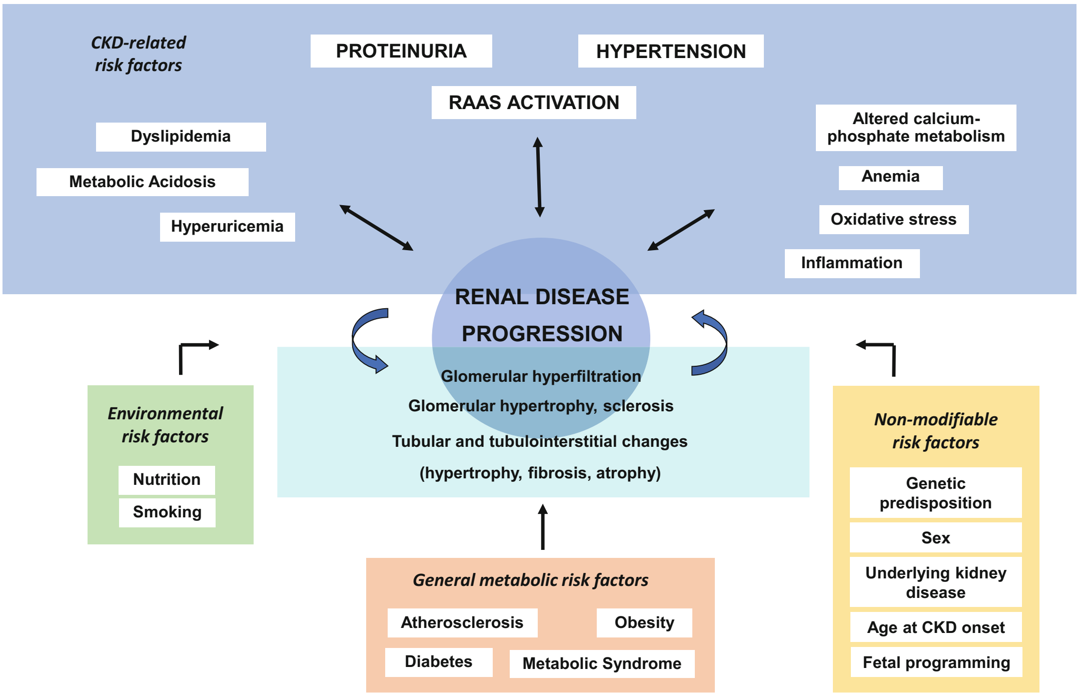
CKD-related risk factors
PROTEINURIA
HYPERTENSION
RAAS ACTIVATION
Dyslipidemia
Metabolic Acidosis
Hyperuricemia
Altered calcium-phosphate metabolism
Anemia
Oxidative stress
Inflammation
RENAL DISEASE PROGRESSION
Glomerular hyperfiltration
Glomerular hypertrophy, sclerosis
Tubular and tubulointerstitial changes
(hypertrophy, fibrosis, atrophy)
Environmental risk factors
Nutrition
Smoking
Non-modifiable risk factors
Genetic predisposition
Sex
Underlying kidney disease
Age at CKD onset
Fetal programming
General metabolic risk factors
Atherosclerosis
Obesity
Diabetes
Metabolic Syndrome

Fig. 3 Nonmodifiable and modifiable risk factors for CKD progression

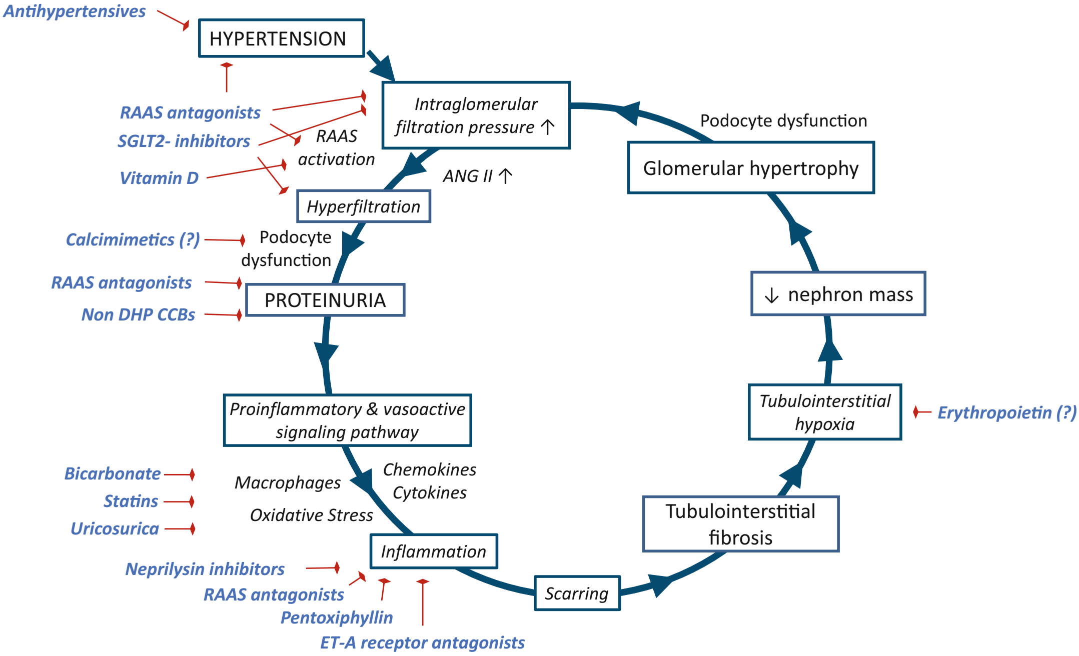
Antihypertensives
HYPERTENSION
RAAS antagonists
SGLT2- inhibitors
Vitamin D
Intraglomerular filtration pressure ↑
RAAS activation
ANG II ↑
Podocyte dysfunction
Glomerular hypertrophy
Hyperfiltration
Calcimimetics (?)
Podocyte dysfunction
RAAS antagonists
Non DHP CCBs
PROTEINURIA
↓ nephron mass
Proinflammatory & vasoactive signaling pathway
Tubulointerstitial hypoxia
Erythropoietin (?)
Bicarbonate
Statins
Uricosurica
Macrophages
Chemokines
Cytokines
Oxidative Stress
Tubulointerstitial fibrosis
Inflammation
Neprilysin inhibitors
RAAS antagonists
Pentoxiphyllin
ET-A receptor antagonists
Scarring

Fig. 4 Pathophysiology and current treatment strategies of hypertension and proteinuria in chronic renal failure. *RAAS* renin-angiotensin-aldosterone system, *non-DHP CCBs* non-dihydropyridine calcium channel blockers, *ET-A* endothelin A, *SGLT2* sodium glucose co-transporter 2, ⊥ inhibitory effect

Treatment of the Underlying Disease

Treatment of the underlying kidney disease remains the optimal approach, although patients often present at a time when irreversible damage has already occurred due to maladaptive repair processes and the resulting fibrosis. However, specific treatment in defined disease entities, for example, complement blockade in C3 complement disorders may prevent further injury and ameliorate CKD progression.

BP Control

In children with CKD, the BP level is correlated with the rate of GFR decline and vice versa; there seems to be an almost linear relationship between BP level achieved by antihypertensive treatment and the rate of kidney failure progression [61]. This relationship appears to persist well into the normal range of BP [34, 62]. Thus, it has been postulated that intensified BP control, aiming for a BP goal in the lower-normal range, might be nephroprotective in CKD patients. However, while several studies in adults have provided proof to the concept that consequent antihypertensive therapy slows down the rate of progression [61], interventional trials comparing different BP targets yielded somewhat conflicting results: A kidney survival benefit of intensified BP control was demonstrated by the MDRD trial in adults with proteinuria and CKD. Patients with proteinuria randomly assigned to a low BP goal (mean arterial pressure <92 mmHg) exhibited improved renal survival after 4 years of follow-up, whereas nonproteinuric patients had no benefit [63]. Extended follow-up for >10 years could demonstrate a persistently reduced renal risk in the low BP group [62].

Nevertheless, this could not be shown in other studies in adult populations [64–66]. In the Ramipril Efficacy In Nephropathy (REIN)-2 extension trial in nondiabetic adults with proteinuria and CKD, a BP target of <130/80 mmHg vs. a diastolic target of <90 mmHg did not result in improvement of renal survival [66]. In the African American Study of Kidney Disease and Hypertension (AASK) trial in >1,000 African Americans with hypertensive nephrosclerosis, randomization to either ramipril, metoprolol, or amlodipine (targeting at mean arterial pressure of 102–107 vs. <90 mmHg) did not affect the rate of GFR loss within 3–6 years [64]. However, intensified BP control exerted a nephroprotective effect during the follow-up period in patients with significant baseline proteinuria and ACE inhibitor treatment [67]. In the Appropriate Blood Pressure Control in Diabetes (ABCD) trial, a low BP target did not improve overall renal survival in hypertensive patients, whereas normotensive patients seemed to have some benefit from lowering BP level to the low-normal range [65]. These partially discrepant findings may be explained by methodological and population differences between studies. Indeed, in a systematic review and meta-analysis including 11 trials providing information on almost 9300 CKD patients, a more intensive BP-lowering strategy reduced the risk of CKD progression and end-stage kidney disease compared to standard BP control. Subgroup analysis showed effect modification by baseline proteinuria; intensive BP lowering was not effective to reduce the risk of kidney failure in patients without proteinuria at baseline [68].

In children with CKD, the ESCAPE trial provided evidence that in children on angiotensin-converting enzyme inhibitors (ACEI) intensified BP control targeting for a 24-h mean arterial pressure below the 50th percentile provides superior long-term nephroprotection compared to a higher target range (50th to 95th percentile) [40]. Despite a relatively modest additional reduction of BP level by around 3–4 mmHg, kidney survival was markedly improved by the intensified intervention protocol. Within 5 years of observation, the risk of losing 50% eGFR or progressing to end-stage kidney disease was 41.7% in the conventional treatment arm compared to 29.9% in the ***strict BP control*** arm [40]. Intensified BP control was effective in children with underlying glomerulopathy as well as renal dysplasia or hypodysplasia, albeit not in other congenital or hereditary nephropathies. Strict BP control was tolerated very well in the vast majority of children.

In view of the results of the ESCAPE trial, the most recent pediatric guidelines published by the European Society of Hypertension have recommended a BP target (for casual BP and for ambulatory BP monitoring) below the 75th percentile in nonproteinuric children with CKD, and below the 50th percentile in those with proteinuria [69]. The AAP Clinical Practice Guideline for Screening and Management of High BP in Children and Adolescents, published in 2017, recommended a BP goal below the 50th percentile for all CKD children [70].

Until publication of the SPRINT (Systolic Blood Pressure Intervention Trial) results [71], most guidelines for BP targets in adult patients with CKD favored treatment to a BP <140/90 mm Hg, with consideration of a lower target of <130/80 mm Hg for those with more severe proteinuria. Because in patients with stage 3 to 4 CKD-intensive BP management seemed to provide the same benefits for reduction in cardiovascular outcome and all-cause mortality as were seen in the full study cohort, a lower target of <130/80 mm Hg for all adults with CKD is now recommended in the USA [72]. The latest European Hypertension Guideline [73] recommends a systolic target BP of 130 to 140 mmHg. Further details on BP management in children is discussed in ▶ Chaps. 62, “Pathophysiology and Epidemiology of Hypertension in Children,” and ▶ 63, “Evaluation and Management of Hypertension in Children.”

Proteinuria Control

Proteinuria is not only a mere marker of progressive CKD but also plays an important causal role in pathogenesis of CKD. This is in line with the finding in interventional trials that reduction of proteinuria is associated with a slowing of GFR loss in the long term [34, 64, 74, 75]. For example, in the MDRD trial, for each 1 g/day reduction in proteinuria observed after 4 months of antiproteinuric therapy (i.e., BP and dietary interventions), the subsequent GFR decline was slowed by about 1 ml/min per year [34]. The goal of antiproteinuric treatment is to reduce proteinuria as much as possible, ideally to <300 mg/m^2/day. This degree of proteinuria reduction appears to be associated with the maximal renoprotective effect [76, 77]. In the ESCAPE trial, ramipril therapy lowered proteinuria by a mean of 44%. Higher proteinuria at baseline was independently associated with a larger proteinuria reduction [41]. In the CKiD cohort, proteinuria was lower in glomerulonephritis patients on RAAS blockers compared to patients who were not on RAAS blockade [38].

In addition, it has been shown, for instance in the MDRD study [34], the ABCD study [78], and the AASK trial [64], that BP control lowered proteinuria. A low BP goal, i.e., <125/75 mm Hg in adults, either reduced proteinuria absolutely by 50% [34] or prevented the two- to threefold increase in proteinuria observed in patients with the more “conventional” BP goal of 140/90 mm Hg [78]. For MDRD study patients with proteinuria >3 g/day, post hoc analysis indicated benefit from treatment to a lower BP goal (<130/80 mm Hg); however, this was related to kidney outcomes only [34].

Many studies of antihypertensive therapy, mostly by ACEI or angiotensin receptor blockade (ARB), suggest that the effect is greatest in patients with high-grade proteinuria [68, 79, 80].

In the ESCAPE trial baseline and residual proteinuria during ACE inhibition was quantitatively associated with kidney failure progression [40, 41]. In addition, the early antiproteinuric effect of ACE inhibition was associated with long-term preservation of renal function. Thus, proteinuria lowering should be considered an important target in the management of pediatric CKD [41]. Of note, whereas the excellent antihypertensive effect of the ACEi treatment and its impact on progression persisted during the ESCAPE trial study period, proteinuria gradually increased again over time, resulting in a level of proteinuria after 36 months that did not differ significantly from that at baseline [40].

With respect to the findings given above, efficient control of BP and minimization of proteinuria are still the two most important measures to preserve residual kidney function.

Additional Nephroprotective Treatment Options

While the different classes of antihypertensive agents are comparable with respect to their BP lowering efficacy, they differ significantly in terms of their effects on proteinuria and CKD progression [64, 75, 81, 82].

By virtue of their pharmacological properties drugs antagonizing the RAAS, i.e., ACEi and ARB, are pharmacotherapeutics of first choice in both adults and children with chronic kidney disease.

RAAS antagonists suppress angiotensin II synthesis (ACEi) or action (ARB). This results in a reduction of intraglomerular pressure and proteinuria, diminished local release of TGF-ß and other growth factors, cytokines and chemokines, with consequently attenuated glomerular hypertrophy and sclerosis, tubulointerstitial inflammation, and fibrosis as well as in a normalized central nervous sympathetic tone by reduced renal afferent nerve stimulation.

Several randomized trials in adults with diabetic or nondiabetic kidney disease have demonstrated a more effective reduction of proteinuria, usually by 30–40%, by ACEi as compared to placebo and/or other antihypertensive agents. In the long term, this was associated with a reduced rate of renal failure progression [74, 76, 80, 83, 84].

ACEis and ARBs are comparable regarding their renoprotective properties. However, the superiority of RAAS antagonists is related to the prevailing degree of proteinuria. Insufficient high dosing of RAAS antagonists may fail to achieve effective RAAS suppression at the kidney tissue level and maximum antiproteinuric and nephroprotective effects. In addition, some patients may develop partial secondary resistance to ACE inhibition or RAAS blockade [85] by compensatory upregulation of ACE-independent angiotensin II production. While the maximal antiproteinuric effects of RAAS antagonists occur at doses exceeding those providing maximal antihypertensive action, regulatory authority approval is usually available only for the indication of hypertension in the respective dose range. Therefore, it is generally recommended to administer these drugs, after confirming tolerability, at their highest approved doses.

The specific effects of RAAS antagonists on glomerular perfusion and intraglomerular pressure induce an immediate drop in the GFR, usually by approximately 10–15% at initiation of treatment. The antiproteinuric response within the first 2–3 months of administration appears predictive of the long-term renoprotective effect [41, 86] and may be utilized to adjust the appropriate drug dose. In patients with CKD, the reduction of intraglomerular pressure may cause a significant increase in serum creatinine, which might be considered a positive predictor of the long-term renoprotective effect [87]. Acute renal failure after administration of a RAAS antagonist is a rare event and usually related to concomitant volume depletion. Thus, in episodes of acute dehydration RAAS antagonists should be temporarily discontinued and hydration ensured to prevent acute kidney injury. Furthermore, in girls who might become pregnant, the teratogenicity of RAAS blockade should be considered and effective contraception has to be provided.

The finding that discontinuation of RAAS inhibitors in children with CKD is associated with an acceleration of kidney function decline, even in advanced CKD [88], underscores the importance of RAAS inhibition in the protection of kidney function.

Dual RAAS blockade using an ACEi and ARB combination therapy has only a minor effect on BP (3–4 mmHg) compared to monotherapy, but increases the antiproteinuric effect by 30–40% [89, 90]. However, findings of the ONTARGET trial in adult populations with high cardiovascular risk did not support dual therapy with telmisartan and ramipril over monotherapy in patients with low GFR or albuminuria [91, 92]. Although combination therapy reduced proteinuria to a greater extent than monotherapy, it had no additional cardiovascular benefit and led to a higher incidence of life-threatening adverse events including acute kidney failure and hyperkalemia, resulting in worse major renal outcomes (chronic dialysis or doubling of serum creatinine). However, it is questionable whether these findings, derived from an adult high-risk population with diabetes and established cardiovascular disease, can be extrapolated to patient cohorts at lower cardiovascular risk [93], for example, pediatric CKD patients.

Mineralocorticoid receptor antagonists (MRA) also act by RAAS suppression resulting in BP lowering. While the use of *spironolactone* is limited by its endocrine side effects, *eplerenone* has minimal affinity for progesterone and androgen receptors and apart from the risk of hyperkalemia, reported side effects were similar to placebo [94]. However, a recent Cochrane review concluded that the effects of aldosterone antagonists when added to ACEi or ARB (or both) on the risks of death, major cardiovascular events, and kidney failure in patients with proteinuric CKD are uncertain. MRA may increase the risk of hyperkalemia, acute kidney injury, and gynecomastia when added to ACEi and/or ARB [95].

In contrast, *finerenone*, a structurally different, nonsteroidal, selective MRA, has been shown in animal studies to block inflammation and fibrosis in both the heart and kidneys with only small effects on serum potassium. In the Fidelio-DKD trial involving adult patients with CKD and type 2 diabetes, treatment with finerenone resulted in lower risks of CKD progression and cardiovascular events than placebo [96]. The simultaneous use of oral potassium absorbers, binding potassium in the gastrointestinal tract, may improve tolerability of MRA in patients with increased risk for hyperkalemia.

The ***renin-antagonist*** *aliskiren*, blocking the conversion from angiotensinogen to angiotensin I, has also been shown to effectively reduce BP to a similar extend as ARBs and a combination therapy of aliskiren and valsartan at maximum recommended doses provided significantly higher reductions in BP than did monotherapy [97]. However, due to the higher risk of cardiovascular complications found in the ALTITUDE study, the combination therapy of aliskiren with ACEi or ARBs in patients with diabetes or reduced renal function is not recommended.

Calcium channel blockers (CCBs) can achieve BP control in CKD patients; however, CCBs of the dihydropyridine type (amlodipine and nifedipine) failed to reduce progression of chronic renal failure. Dihydropyridine CCBs may even induce or aggravate proteinuria and more rapid CKD progression [75] and should be avoided in patients with proteinuria unless in combination with RAAS antagonists [98].

β-blockers have been shown to be also effective in lowering BP in CKD patients. Metoprolol and atenolol demonstrated beneficial effects on the decline of renal function in CKD patients [99] and in the AASK trial metoprolol had an antiproteinuric effect almost comparable to ramipril [64]. Newer β-blockers such as carvedilol have even improved antiproteinuric effects, probably due to sympathicoplegic effects.

Because hypertension is a multifactorial disorder, monotherapy is often not effective in controlling BP and proteinuria. In the ESCAPE trial one-third of patients required ***antihypertensive combination therapy*** to attain a BP level in the normal range. For those patients with a target BP in the lower normal range (<50th percentile) antihypertensive combination therapy had to be prescribed in almost 40%. This is in line with findings from the CKiD study where 35% of patient had combination therapy at baseline [100].

In CKD patients, RAAS antagonists are most commonly combined with a diuretic or with a calcium channel blocker, whereas their combination with a β-blocker usually does not exert an additive effect on BP control.

In view of the fact that nocturnal BP non-dipping is an independent risk factor for CKD progression, ***antihypertensive chronotherapy***, i.e., the timing of antihypertensive drug dosing, may be relevant. While antihypertensive “chronotherapy” has not been demonstrated to affect CKD progression as yet, in a study randomly assigning hypertensive CKD patients either to take all prescribed antihypertensive medications in the morning or to take at least one of them at bedtime, bedtime dosing was associated with improved BP control and significant reduction of cardiovascular risk [101].

Over the last decade, several drugs developed to improve glucose control in patients with type 2 diabetes mellitus (T2DM), such as metformin, glucagon-like peptide 1 (GLP-1) agonists, dipeptidyl peptide 4 (DPP-4) antagonists, and ***sodium glucose cotransporter 2 (SGLT2) inhibitors,*** have been shown to exert advantageous cardiorenal effects. Among these substances, SGLT2 inhibitors (gliflozins) are the most promising drug class. Apart from the glucose lowering effects, SGLT2 inhibitors also decrease BP, body weight, and albuminuria. Large clinical outcome trials with the SGLT2 inhibitors dapagliflozin, canagliflozin, or empagliflozin in T2DM have demonstrated potent long-term renoprotective effects of SGLT2 inhibition, including reductions of 30–50% in albuminuria or proteinuria and clear beneficial effects on hard renal outcomes such as doubling of serum creatinine, eGFR decline, attainment of KRT, or death due to renal disease [102, 103]. These nephroprotective effects are based on a decrease of sodium delivery to the distal tubule and reduced glomerular blood flow leading to reversal of glomerular hypertension and hyperfiltration [104].

Prevention of CKD Progression by Optimized CKD Treatment

Treatment of uremic dyslipidemia may be another component of renoprotective therapy. General measures in the prevention of dyslipidemia in CKD patients include prevention or treatment of malnutrition, correction of metabolic acidosis [47], hyperparathyroidism [46], and anemia [105], all of which may contribute to dyslipidemia. Although, as for the general population, therapeutic life style modification (diet, exercise, and weight reduction) is recommended for adults and children with CKD-related dyslipidemia by the KDIGO Clinical Practice Guideline for Lipid Management in Chronic Kidney Disease [106], the ***lipid-lowering*** effect of these interventions in CKD patients is usually not impressive. Thus, in adults with CKD stage 3–5 and hypercholesterolemia, either aged $>$ 50 years or aged 18–50 years plus increased cardiovascular risk treatment with a statin or statin/ezetimibe combination is recommended. However, in children treatment of CKD-related hypercholesterolemia is not recommended and treatment for hypertriglyceridemia should focus on severe cases due to lack of clear benefits compounded by safety concerns associated with long-term use in children [106].

With respect to renoprotection, experimental evidence suggests that statins may retard renal disease progression not only by their lipid lowering, but also by lipid-independent pleiotropic effects. Data from a meta-analysis including 10 studies on the impact of statin therapy on CKD progression add evidence that statin use in CKD patients may delay progression, but may have a dose-related effect on kidney function as only high-intensity statin therapy significantly improved renal function assessed by estimated GFR [107].

Also, the degree of CKD and proteinuria may influence the effect of statin therapy on CKD progression. A study in almost 3500 CKD patients demonstrated statistically significant effects on GFR decline in adults with CKD stage 3b to 5 or proteinuria $>$ 1000 mg/day, while no significant effects were seen in mild-to-moderate CKD and nonproteinuric patients [108].

No studies have evaluated the usefulness of statins in CKD children to date.

Observational studies report independent associations between ***serum phosphate level, PTH, FGF-23, klotho, and vitamin*** D and the risk of renal disease progression, cardiovascular disease, and mortality; this is discussed further in ▶ Chap. 72, “Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD).”

In animal models, vitamin D (paricalcitol) effectively attenuates renal interstitial fibrosis through a combination of inhibitory actions on the RAAS, inflammation, and epithelial/mesenchymal transition [109]. In children with CKD, 25(OH)D levels $>$50 nmol/L were associated with better preservation of renal function, even in the presence of concomitant ACEi therapy [110].

In this context, a PTH-independent beneficial effect of **phosphate restriction** on kidney disease progression has been suggested by animal studies. The finding that dietary phosphorus restriction may stabilize kidney function has been also confirmed by several studies in adults with CKD [63]. Additionally, calcium-free phosphate binders may have superior reno- and cardioprotective advantages in patients with CKD. For instance, sevelamer has been proven to have a lower all-cause mortality in patients with CKD stage 3 to 5D compared to calcium-based phosphate binders [111].

Metabolic acidosis is common in patients with CKD and may contribute to the development and worsening of proteinuria and tubulointerstitial fibrosis. In a randomized controlled trial evaluating the renoprotective potential of oral bicarbonate supplementation in adult patients with CKD [112], CKD progression rate was reduced to 1 ml/min/year in patients with serum bicarbonate levels ≥22 mmol/L compared to >2.5 ml/min/year in patients with uncorrected low bicarbonate levels. Similarly, data from the 4C Study provide evidence that children with time-varying serum bicarbonate under 18 mmol/l have a significantly higher risk of CKD progression compared to those with a serum bicarbonate of 22 or more mmol/l [53]. Thus, control of metabolic acidosis may be an important component of renoprotective therapy in patients with progressive CKD.

Another factor in progressive CKD is serum ***uric acid***. Allopurinol treatment improved renal outcome in CKD patients, compared to placebo, without a change in BP level [113] and Febuxostat treatment has been shown to slow down kidney disease progression [114]. However, the CKD-FIX trial could not demonstrate a beneficial effect of uric acid lowering on kidney disease progression in stage 3–4 CKD patients [115].

Much attention has been focused on the effect of ***dietary protein restriction*** on progression. Based on prior animal studies, the MDRD study sought to determine whether less than 0.6 g/kg/day protein intake would delay the onset of KRT. Although early results suggested some benefit [116], a systematic analysis by the Cochrane Group identified only two adequate studies of dietary protein intake and did not find a beneficial effect of this treatment [117]. In children reduced protein intake to the maximal acceptable lower limit did not slow down renal progression [27]. Further reductions may be effective but not acceptable for patients. Furthermore, therapeutic strategies of protein reduction in children may be conflicting, since a low-protein diet carries the risk of low-calorie intake, while a high-calorie intake is needed for optimal growth. Therefore, a low-protein diet is not recommended.

Other Potential Treatment Options

While RAAS antagonists exert indirect anti-inflammatory and antifibrotic properties via blockade of RAAS-dependent profibrotic and inflammatory pathways as outlined above, other nonspecific ***anti-inflammatory and antifibrotic drugs*** may decrease fibrogenesis by reducing macrophage infiltration and subsequent recruitment of fibroblasts or generation of reactive oxygen species. For example, pirfenidone, exerting antifibrotic, antioxidant, and anti-inflammatory properties, interfering with the expression, secretion, and the effect of TGF-β, improved kidney function and proteinuria and reduced kidney scarring in animal models; however, in clinical trials in FSGS or diabetic nephropathy patients, pirfenidone showed conflicting results failing to prevent proteinuria or GFR decline particularly in patients with renal dysfunction [118, 119].

Activation of ET_A receptor mediates inflammation, fibrosis, and proteinuria, while ***endothelin A receptor antagonists*** (ERAs) have been shown to reduce albuminuria and BP, but also to cause sodium retention and anemia. A large randomized controlled trial in patients with T2DM and CKD using the fairly unselective endothelin receptor antagonist avosentan was terminated early because of an increased frequency of heart failure with avosentan [120]. Atrasentan, a more selective endothelin receptor antagonist, reduced the risk of renal events compared with placebo in preselected patients with diabetes and CKD. These data support a potential role for selective endothelin receptor antagonists in protecting renal function at least in patients with T2DM at high risk of developing ESKD [121]. Combined ET_A and angiotensin II blockade by sparsentan, a drug comprising moieties of irbesartan and atrasentan in one molecule, has been shown to be effective in patients with primary FSGS in achieving significantly greater reductions in proteinuria compared to irbesartan [122].

Pentoxifylline (PTF), a nonspecific phosphodiesterase inhibitor, shows anti-inflammatory, antiproliferative, and antifibrotic properties. Small clinical trials evaluating PTF in patients with CKD reported conflicting data. A meta-analysis of studies on PTF in combination with ACEi or ARB treatment provided evidence that combination therapy may lead to a greater reduction in proteinuria and attenuate the decline in eGFR in patients with stages 3–5 CKD [123]. Of note, the existing data suggest that patients with proteinuria may have better protection from PTF treatment than nonproteinuric patients.

Neprilysin (neutral endeopeptidase or membrane metallo-endopeptidase) degrades numerous peptide hormones, among others bradykinin, endothelin, ANP, and BNP. ***Neprilysin inhibition*** (NEPi) may be a therapeutic strategy that enhances the activity of the natriuretic peptide system inducing natriuresis, diuresis, and inhibition of the renin-angiotensin system and the sympathetic nervous system. Sacubitril/valsartan, the first angiotensin receptor-neprilysin inhibitor (ARNI), has been shown to substantially improve not only cardiovascular outcomes in adults with heart failure but also to delay CKD progression in this population. Although ARNIs have not shown similar effects on kidney function in the short-to-medium term in CKD patients, treatment was associated with substantial reductions in cardiac biomarkers and in BP [124].

Conclusions

In children with congenital or acquired kidney diseases progression towards KRT is common and once a significant impairment of kidney function has occurred progression continues irrespectively of the underlying kidney disease. Timing of onset of CKD and progression rate in defined disease entities may be influenced by genetic factors. Hypertension and proteinuria are the most important independent risk factors for renal disease progression. Therefore, therapeutic strategies to prevent progression should target BP control and reduction of proteinuria. Blockade of the RAAS exerts not only BP lowering but also antiproteinuric and anti-inflammatory effects, thus attenuating renal disease progression. Furthermore, intensified BP control exerts additional renoprotective effects in pediatric CKD.

Other important factors contributing to renal disease progression comprise mainly the secondary complications of CKD, such as metabolic acidosis, anemia, dyslipidemia, hyperuricemia, and vitamin D deficiency. Thus, optimal treatment of CKD-associated disorders is key in preventing CKD progression.

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Epidemiology and Management of Chronic Kidney Disease in Children

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Elaine Ku and Jérôme Harambat

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Abstract

The global burden of chronic kidney disease remains high, and the cost of caring for patients with CKD is substantial, especially if end-stage kidney disease occurs. The incidence and prevalence of chronic kidney disease is understudied in children, and most of our data and current understanding are derived from large national registries of children receiving kidney replacement therapy. We have made significant progress in identifying risk factors that lead to the progression of chronic kidney disease in children, which has led to improved outcomes for children who require kidney replacement therapy. However, not all children have access to dialysis and kidney transplantation when end-stage kidney disease occurs depending on where they live in the world. Understanding the epidemiology of chronic kidney disease in children remains critical for the early recognition and prevention of the progression of chronic kidney disease and achieving equity in outcomes globally.

Keywords

Chronic kidney disease · Estimating glomerular filtration rate · End-stage kidney disease · Epidemiology · Progression of CKD

Introduction

As of 2017, approximately 9% of the world's population is estimated to have chronic kidney disease (CKD), and the global burden of disease remains high [1]. The cost of caring for patients with CKD remains substantial, and some developing countries do not have the resources to provide kidney replacement therapy (KRT) to those in need. While CKD is a major cause of morbidity and mortality [2], the prevalence of CKD remains understudied in certain areas of the world and in children. Much of our current understanding of the epidemiology of CKD in children is extrapolated from large national registries of children receiving KRT [3–5], as few countries routinely screen healthy children for the presence of CKD [6]. As a consequence, limited data are available surrounding the prevalence of CKD in children.

Despite advances in the care of children with CKD over the past 30 years, a small proportion of children with CKD still progress to end-stage kidney disease (ESKD) and require KRT during childhood [2]. The onset of ESKD is particularly devastating for children, as ESKD can have substantial adverse effects on growth, psychosocial and emotional well-being, educational attainment, and patient-centered outcomes including quality of life [7]. Understanding the epidemiology of CKD in children is critical for the early identification and detection of disease and delivery of interventions to either prevent or slow the progression of CKD. In addition, knowledge of the presence of CKD allows for the timely preparation of affected children and their families for KRT.

Definition of CKD in Children and Measurement of Kidney Function

According to the Kidney Disease Improving Global Outcomes (KDIGO), CKD in children $\geq$2 years of age is defined as either [1] presence of an glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for at least 3 months or [2] presence of structural damage or markers of reduced kidney function, such as proteinuria, albuminuria, or histologic abnormalities even when the GFR is $\geq$60 mL/min/1.73 m^2 [8]. However, in children under 2 years of age whose GFR is still undergoing expected physiological increases to adult levels, this definition of CKD is not applicable. Normative age-specific reference ranges for serum creatinine values in children under 2 years of age are available [9]. The degree of impairment in kidney function in children under 2 years of age can be determined based on the number of standard deviations that any serum creatinine value is below the mean value that is expected for age.

In children, the GFR can be estimated using the revised (bedside) Schwartz equation, which is computed as 0.413*serum creatinine/height (in cm) [10]. The bedside Schwartz equation is currently the most commonly used creatinine-based equation for the estimation of GFR in clinical practice. The bedside Schwartz equation has been validated in various pediatric populations across a wide range of kidney function, although validation studies in children with very low kidney function (e.g., GFR < 15 mL/min/1.73 m^2) are limited [11, 12]. Other equations are also available for the estimation of kidney function in children [13, 14].

For young adults, some studies have suggested that using a mean of the values obtained from the bedside Schwartz equation and CKD-EPI creatinine-based equation (which has been validated in adults) improves the accuracy of the estimation of kidney function [15, 16].

Because creatinine is influenced by muscle mass and children with CKD may have low muscle mass and growth impairment, biomarkers that are not influenced by muscle mass such as cystatin C may also be useful for the estimation of kidney function. Equations to estimate kidney function using cystatin C alone or cystatin C in conjunction with serum creatinine are available and have been validated in children [17, 18]. Because these equations require the incorporation of sex, height, cystatin C, and blood urea nitrogen, their use may be less practical than the bedside Schwartz equation, and cystatin C assays may not be available in all parts of the world.

CKD is classified into stages of severity based on the level of GFR and proteinuria in children in the same approach as in adults. Currently, KDIGO guidelines recommend the consideration of albuminuria for staging of the severity of CKD, as adult studies have shown that presence of albuminuria is highly predictive of progression of CKD, and albuminuria measurements can be standardized and more precisely measured at lower ranges than proteinuria [8, 19, 20]. In children, proteinuria (often expressed as a urine protein-to-creatinine ratio) is more often used as a marker of CKD progression, as some etiologies of CKD in children do not necessarily lead to albuminuria but can cause non-albuminuric proteinuria. In children, proteinuria has been shown to have similar prognostic implications as albuminuria, and reduction of proteinuria is associated with better kidney survival as observed in adults [21–23]. A recently developed equation that converts proteinuria to albuminuria is available [24], but has yet to be externally validated.

Incidence and Prevalence of CKD

Screening for CKD remains a matter of controversy, and most countries no longer perform routine screening for the presence of CKD except in Asia [25, 26]. Spain estimated the incidence of CKD stages 2–5 in children was approximately 9 per million children per year and prevalence was 71 per million children per year [27]. In a study in England, the incidence and prevalence of children was similar to that reported in Spain but increased over time to 25 and 105 per million children per year, respectively [28].

In a recent study from National Health and Nutrition Examination Survey in the US, between 3–4% of the screened adolescent population had albuminuria, and $< 1\%$ had low eGFR that could potentially be consistent with presence of CKD stage 3, although confirmation of abnormal eGFR was not performed in this study [29]. However, these rates are consistent with the prevalence of low eGFR and albuminuria reported in children in population-based studies from China and Turkey [30, 31]. International variations in the incidence of CKD and its prevalence are shown in Fig. 1.

A recent study of Israeli adolescents and young adults who had a health screening exam prior to their compulsory military service between 1967 and 1997 and who reported a history of congenital abnormalities of the kidney and urinary tract (CAKUT), glomerulonephritis, or pyelonephritis were more likely to develop ESKD during

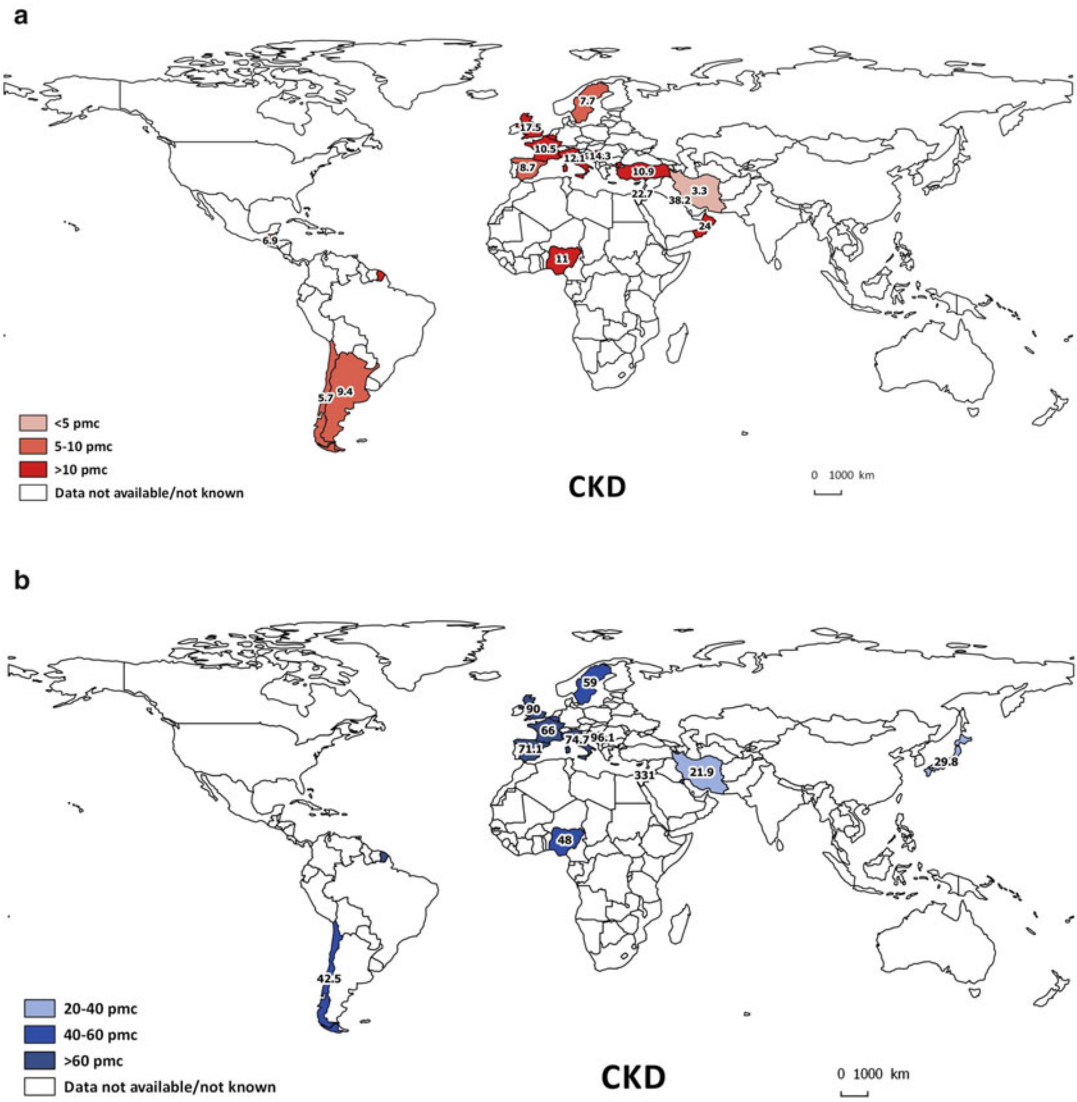
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Fig. 1 (**a**) Reported incidence (pmc) of CKD (age 0–16 to 0–18 years depending on the studies and variable period and definition of CKD) in selected countries worldwide. (**b**) Reported prevalence (pmc) of CKD (age 0–16 to 0–18 years depending on the studies and variable period and definition of CKD) in selected countries worldwide

adulthood, and their ESKD onset was at a young age during 30-years of follow-up [32]. The higher risk of ESKD in these Israeli individuals was noted despite the presence of normal serum creatinine, blood pressure, and absence of dipstick proteinuria at the time of their health screening exam [32].

In a similar study, presence of persistent and asymptomatic hematuria in Israeli adolescents and young adults was associated with a higher albeit small absolute risk of ESKD onset during later adulthood [33]. These data suggest that a small proportion of the general population of children could have asymptomatic CKD that could be diagnosed earlier if mass screening were to be implemented, but no studies have shown that early detection of asymptomatic disease would improve long-term outcomes.

Incidence and Prevalence of ESKD in Children

Data on pediatric ESKD requiring KRT (dialysis or kidney transplantation) are more robust due to the availability of national or international registries, at least in developed countries in Europe, Australia and New Zealand, and the USA. However, in some countries, access to KRT for children remains limited, and the vast majority of children receiving KRT are those residing in developed countries [34].

The incidence of ESKD in children varies widely across different countries but ranges from 5 to 10 per million children per year among patients under 20 years (Fig. 2) [5, 35, 36]. The incidence of ESKD in Japan is slightly lower than that reported in other nations, whereas the incidence in the USA is higher than that reported in other nations [37, 38]. The incidence of ESKD varies by race (higher in Blacks, Aborigines, and Southeast Asians compared with Caucasian children) [39] and age (children <5 years of age and adolescents have higher incidence). [38, 40] The incidence of ESKD has remained stable in some nations but is declining in the US [38].

The prevalence of ESKD in children varies between 40 and 60 per million children in Europe and Australia [41, 42], with the prevalence being higher in the USA (around 100 per million children) and lower in Brazil [38]. This prevalence has remained relatively stable in most nations since the 2000s (Fig. 2b).

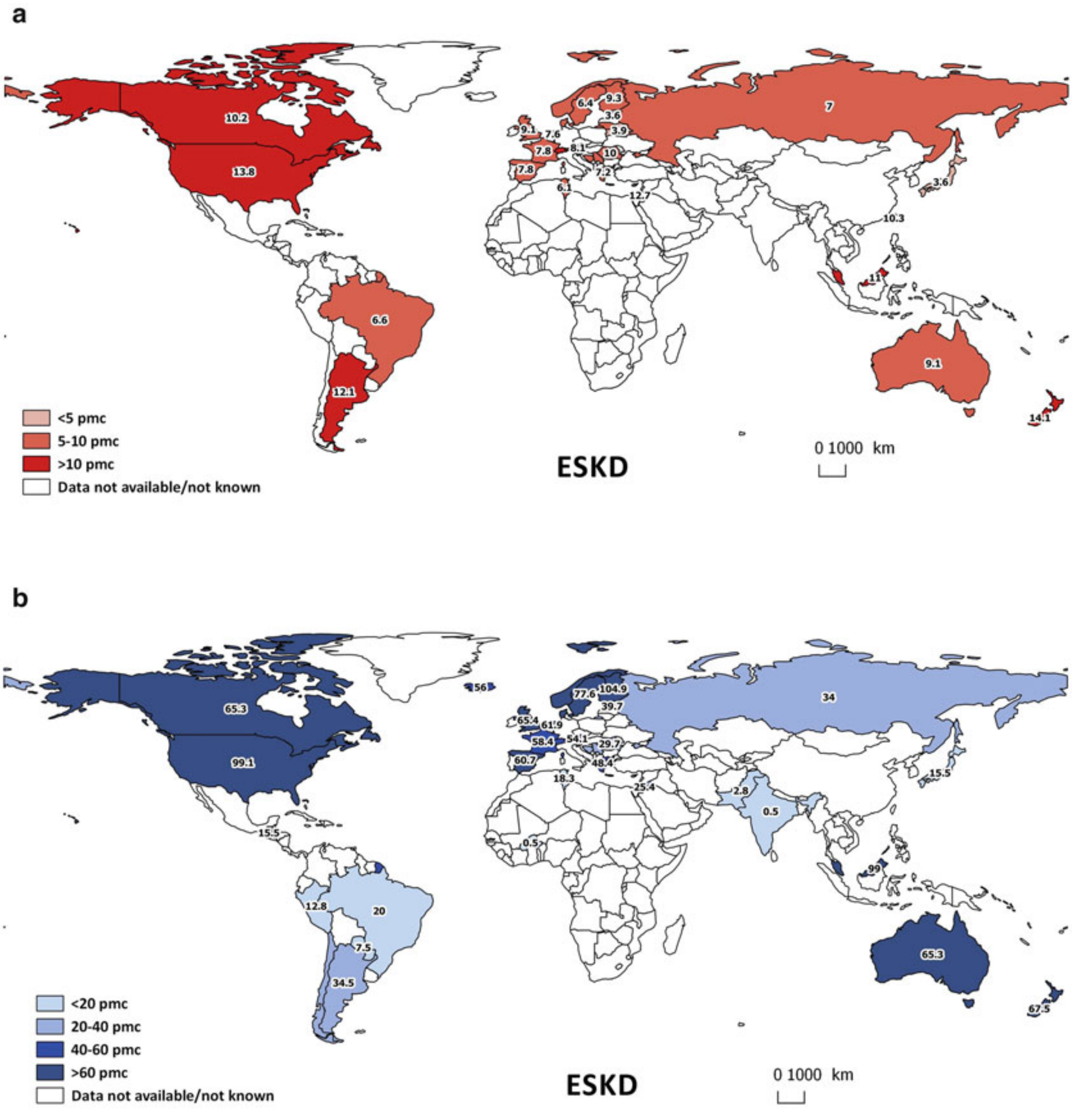
Etiology of CKD and ESKD in Children

The etiology of CKD in children differs from that observed in adult populations, where diabetes and hypertension remain the leading causes of kidney disease. In children, CAKUT account for 50–60% of CKD in children, followed by glomerular diseases (5–15%) and hereditary conditions (10–20%) [5]. More recently, the lower cost and enhanced capacity to perform whole exome sequencing in children with CKD has led to increased recognition of genetic (and specifically monogenic) causes of CKD, which is now thought to account for 20% of all CKD prior to the age of 25 years [43]. However, the classification of different etiologies of CKD (such as the exact definition of CAKUT) can vary across different studies, which may lead to variations in the reported prevalence of the different causes of CKD.

Of note, the prevalence of different causes of CKD varies substantially by ethnicity and geographic location [3, 28, 44–46, 48, 49]. For example, congenital nephrotic syndrome of the Finnish type is the leading cause of pediatric ESKD in Finland but is a rare cause of ESKD in other nations. In addition, presence of two high-risk variants of *APOL1* genotype (which is more common in the Black population due to the protection conferred by two high-risk variants of *APOL1* against trypanosomiasis) may contribute to a higher prevalence of CKD in children in certain parts of the African continent and in the Black population [49]. High-risk *APOL1* genotype has been associated with glomerular disease (especially focal segmental glomerulosclerosis) and HIV-associated nephropathy in children [50, 51].

In low- and middle-income countries that do not have national registries, estimating the most common causes of CKD is significantly more challenging and based primarily on single-center data [52–55]. Endemic causes of CKD in certain parts of the world such as Central America (Meso-American nephropathy) or Sri Lanka may be an important cause of CKD in children and young adults, though the lack of routine screening and performance of kidney biopsies may complicate the ability to obtain accurate estimates of the prevalence of such etiologies of CKD in children [56, 57].

Among children who develop ESKD, the prevalence of various etiologies of kidney disease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Data not available/not known
ESKD
0 1000 km

Fig. 2 (**a**) Incidence of KRT per million children (pmc) of the same age. Data of pediatric patients aged 0–19 years except USA (0–21 years) and United Kingdom (0–15 years). Incidence rates of 2018 except Brazil (2012), Israel (2017), Malaysia (2015), Russia (2017), Taiwan (1998–2009), Tunisia (2017), and USA (2016). Incidence rates of dialysis only for Argentina, Brazil, Israel, Japan, and Singapore. (**b**) Prevalence of KRT per million children (pmc) of the same age. Data of pediatric patients aged 0–19 years except USA (0–21 years) and United Kingdom (0–15 years). Prevalence rates as of December 31, 2018 except Brazil (2012), Guatemala (2016), Malaysia (2015), Russia (2017), Tunisia (2017), and USA (2016). Prevalence rates of dialysis only for Argentina, Brazil, Guatemala, Japan, Singapore, and Turkey

differs from that observed in children with CKD. CAKUT and glomerular diseases remain the most common causes of kidney disease in the pediatric ESKD population, but the prevalence of CAKUT as a cause of ESKD is lower than for CKD and ranges from 25–35% in most reported studies due to the slower progression of non-glomerular diseases towards ESKD [38, 58].

Kidney Replacement Therapy Modalities

Approximately 20% of children start KRT with pre-emptive transplantation in Europe and the United States, although the rates of pre-emptive transplantation vary by race and geographic location [59–61]. In some countries, kidney transplantation is not available as an option for children [34]. Children who do not receive pre-emptive kidney transplantation may receive peritoneal dialysis or hemodialysis, but the prevalence of the use of each of these modalities varies substantially depending on the country, reimbursement system for ESKD care, and public policies [62, 63]. The most common modality in most countries is hemodialysis, although in younger children less than 5 years of age, peritoneal dialysis is a more prevalent treatment modality.

In terms of prevalence of treatment modalities for ESKD, kidney transplantation is by far the most common treatment modality in industrialized nations, though disparities in access to kidney transplantation and living donors do exist by race, sex, and across different nations [64–66]. Differences between countries, particularly in Europe, have been attributed to factors such as whether pediatric priority is considered in the organ allocation system, acceptance of and practices related to deceased organ donation, availability of living donors, and payor and public policies surrounding kidney transplantation [64, 67].

Epidemiology Surrounding Risk Factors for the Progression of CKD and CKD Management

The progression of CKD varies substantially depending on the etiology of CKD, the severity of kidney impairment, and the presence of additional modifiable or non-modifiable risk factors or genetic susceptibility. Data from one US cohort of children suggested that the progression rate to ESKD was 17% at 1 year and 39% at 3 years, and median time to progression to ESKD was 4.5 years in a cohort of 4000 children with stage 2–4 CKD [68]. However, the rate of progression to ESKD varies substantially based on the presence or absence of additional risk factors, most importantly of which are the level of kidney function and degree of proteinuria [69].

Non-modifiable Risk Factors for the Progression of CKD to ESKD

There are multiple risk factors for the progression of CKD in children that are not readily modifiable. First, the etiology of CKD is important, as children with glomerular diseases have been shown to have faster progression of disease compared to children with non-glomerular etiologies of CKD [70]. For children of Black race, the presence of two variants of *APOL1* genotype has been associated with faster progression of CKD [50–52, 71]. Although presence of two high-risk variants of *APOL1* is strongly associated with progression of disease, to date, no specific therapeutic interventions have been shown to delay the progression of CKD in such individuals.

Preterm birth and low birth weight have also been associated with onset of faster eGFR decline or development of kidney disease, which in part may be due to lower nephron mass [72–75]. Some children may have genetic loci of susceptibility to the progression of CKD [76]. Parental literacy has been identified as a risk factor for progression of CKD in children [77]. While in adults, low socioeconomic status has also been a predictor of progression of CKD, in children, this relationship has not been as strongly established. In one study, annual household income was not associated with progression of CKD in children [78].

Modifiable Risk Factors for the Progression of CKD to ESKD

Proteinuria is one of the strongest risk factors for the progression of CKD in adults and children [22, 23, 70, 79]. Suppression of proteinuria by an angiotensin-converting enzyme inhibitor was strongly predictive of kidney survival in participants of the ESCAPE trial [23]. These data align closely with data from adults with CKD, in whom suppression of proteinuria is also a target for slowing the progression of CKD, especially in adults with diabetes [8]. In general, use of renin-angiotensin-aldosterone system blockade is recommended in order to reduce proteinuria in children and adults with CKD.

There is a high prevalence of masked hypertension in children with CKD which has been associated with target organ damage [80], and routine ambulatory BP monitoring on a regular basis is recommended for the initial diagnosis of hypertension and to ensure adequate BP control in children. One of the only randomized controlled trials in children with CKD, the ESCAPE trial demonstrated that intensive lowering of ambulatory blood pressure below the 50th percentile mean arterial pressure for age was associated with slower progression of CKD to KRT over 5 years of trial follow-up [81]. This has led KDIGO guidelines to recommend intensive lowering of ambulatory BP to <50th percentile [82]. However, targets for clinic BP treatment in children with CKD remain a subject of debate, as the correlation between ambulatory and clinic BP is not well established.

Some studies have suggested that obesity is a risk factor for the development of ESKD in children as in adults [83, 84]. Although failure to thrive can occur in children with CKD, a growing prevalence of obesity has been noted in children starting ESKD [85, 86]. In one study of more than one million Israeli young adults undergoing military health screening exam, elevated BMI (meeting criteria for overweight or obese) was associated with a higher risk of diabetic and non-diabetic ESKD during long-term follow-up, although the overall absolute incidence of ESKD was very low in this cohort [84]. Few longitudinal studies have examined whether changes in weight (or weight modification) can alter the rate of progression of CKD. In one observational study, weight loss (but not weight gain) was associated with higher risk of onset of the need for KRT than maintenance of stable weight [87]. Obesity in children starting KRT has been associated with a substantially higher risk of mortality during long-term follow-up [86].

Anemia is common in children with CKD and likely multi-factorial [88]. The presence of anemia in children with CKD has been associated with a more rapid decline in kidney function as well as an increased risk for hospitalization [89, 90]. Currently, higher hemoglobin targets are recommended in children with CKD or ESKD compared with recommended targets in adults, as these higher targets have not been associated with adverse cardiovascular events or death as observed in adults [91, 92].

Finally, presence of metabolic acidosis, hyperuricemia, and low serum albumin are additional risk factors that have been associated with progression of CKD in children [68, 95, 96, 98, 99].

The management of specific risk factors that may help delay the progression of CKD are reviewed in subsequent chapters. An overview of the management of CKD risk factors is shown below in Table 1.

Table 1 Overview of the treatment of select risk factors to delay the progression of kidney disease

Risk Factor	Recommendation
Hypertension	Lower blood pressure to <50th percentile ambulatory mean arterial pressure
Proteinuria	Use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers to achieve suppression of proteinuria
Metabolic acidosis	Treatment with alkali to maintain bicarbonate levels >22 meq/L
Anemia	Target hemoglobin is 11–12 g/dL in children Treat iron deficiency if present Treat with erythropoietin-stimulating agents
Weight	Maintenance of normal weight status is recommended Collaboration with a dietician to ensure adequate caloric intake is recommended
Height	Maintenance of normal height is recommended Correction of anemia, metabolic acidosis, and abnormalities in bone mineral disease

Predicting the Risk of Progression of CKD

In recent years, although there has been a trend towards earlier initiation of dialysis in both adults and children, this has not been associated with better outcomes in children starting KRT [97]. There has been recent interest in prediction models that can better guide clinicians in appropriately timing discussions surrounding KRT and preparation of children and their families for such. In adults, the Kidney Failure Risk Equations were developed for the prediction of a 2- and 5-year risk of progression to KRT and were validated in a large multi-national cohort with high risk discrimination [98]. The Kidney Failure Risk Equations were also validated in a cohort of children with CKD and found to have similar high risk discrimination [98, 99]. This calculator's advantages include its simplicity, as only four variables (age, sex, eGFR, and proteinuria) are needed to estimate the risk of developing ESKD over defined periods of time. A second risk prediction model was recently developed in children with CKD and provides an estimate of the time to progression to KRT [69]. This calculator incorporates cause of ESKD, kidney function, and proteinuria to provide estimates of the remaining time before the need for KRT arises.

In addition, novel biomarkers for the progression of CKD in children have been identifies. For example, plasma soluble urokinase plasminogen activator receptor (SUPAR) has been shown to be a biomarker of CKD progression, even after accounting for kidney function [100, 101]. However, whether treatment should be modified in relationship to SUPAR levels remain unclear. Fibroblast growth factor-23 which is a key player in abnormal mineral bone metabolism pathways in CKD has also been identified as an independent predictor of CKD progression, though whether FGF-23 is a therapeutic target remains unclear [93].

Complications of CKD

Various complications of CKD are discussed at length in the relevant chapters. A brief overview is provided here.

Cardiovascular Disease

The life expectancy of a child who develops ESKD is substantially shorter than that in the general population without ESKD [102]. Young adults treated with dialysis have mortality rates that are more than 100-fold higher compared with their healthy age-matched peers [103]. The leading cause of death among children and adults with ESKD has been attributed to cardiovascular disease (CVD), and in particular, sudden cardiac death [104, 105].

Children with CKD are likely to have many traditional cardiovascular risk factors, including hypertension and dyslipidemia [106, 107]. However, children with CKD and ESKD may also share many non-traditional risk factors for cardiovascular disease, including volume overload, inflammation, and abnormalities of parameters in the bone mineral metabolism [108–110]. There is ample data to suggest that children with CKD have subclinical cardiovascular disease. Elevated carotid intimal thickness and pulse wave velocity have been noted in children with CKD [111]. As CKD progresses, the prevalence of left ventricular hypertrophy has also been shown to increase [111, 112]. While the absolute rate of atherosclerotic events remains low in children [113], adolescent and young adults with ESKD are both at risk for hospitalization for cardiovascular-related complications [103].

Growth

Many children with childhood-onset CKD will have short stature, which is likely related to a number of nutritional and metabolic factors including anemia, uremia, metabolic acidosis, derangements in calcium, phosphorus, parathyroid hormone, and hormonal disturbances [111, 115]. Growth failure has been associated with a higher risk of hospitalizations and mortality in children with ESKD [116, 117].

The clinical management of growth retardation in children with CKD includes correction of metabolic derangements including acidosis and mineral and bone disorder and careful attention to the adequacy of nutrition [118]. Recombinant human growth hormone (GH) is a treatment option, though under-utilization of GH is common and GH may not be available in all countries [119]. Height gain and GH use have been associated with higher physical and social functioning as reported by children and parents, but the need for daily injections may increase the likelihood of nonadherence to therapy [120].

Mineral Bone Disease

Abnormalities in calcium and phosphorus homeostasis and parathyroid hormone are also common in children with CKD, with elevated serum phosphorus and low calcium often observed starting in stage 4–5 CKD. Dietary modification is the first-line treatment for such abnormalities. Phosphate binders and vitamin D supplementation are mainstays of therapy in order to normalize mineral and bone disorder parameters, which are important for growth and bone health [121]. Such abnormalities should be corrected prior to the consideration of GH therapy. Although normalization of calcium and phosphate parameters can be achieved, treatment has not been consistently associated with improved survival. Fractures have been reported in 16% of children with CKD in one US cohort [122].

Neuro-Cognition

Children with CKD are at increased risk for neurocognitive deficits compared to their healthy peers. CKD-related factors that may contribute to neurocognitive changes of CKD include anemia, hypertension, uremia, and cardiovascular disease. In general, children with CKD have been observed to have mild deficits in academic skills, memory, visual motor performance, and executive function, with such deficits being worse in children treated with dialysis than with CKD [123–125]. Younger age of CKD onset and longer duration of disease have been associated with lower IQ and greater severity of neuro-cognitive deficits [126, 127].

Quality of Life

Life participation, survival, kidney function, and infection are some of the most important concerns for children and adolescents with CKD or ESKD and their families. Children with CKD have reported poorer overall quality of life compared to healthy children, including lower physical, emotional, and social functioning [128]. Similar poor quality of life has been reported in children and adolescents receiving KRT [129]. Not only does CKD affect the child, but families have also reported significant stressors, including inability of parents to maintain employment, psychological fatigue, and inability of parents to balance their personal needs with the needs of their child with chronic illness, and financial stressors related to the care of a child with CKD [130–132].

Transition of Children with CKD or ESKD into Adulthood

Adolescents are at higher risk for complications related to CKD, as self-management of CKD or ESKD and non-adherence to therapy is frequently present during this tumultuous period. Non-adherence in children with CKD has been reported to range from 4% to 22% [133]. In one study, patients 14–16 years old were reported to have the highest rates of graft loss among all patients $\leq$55 years old [134]. Children who survive to adulthood with KRT have reported lower physical functioning and quality of life and are less likely to be employed, have started families, and have high-income jobs [135].

Careful attention to the transition of children with CKD or children receiving KRT into adult healthcare systems is recommended in order to prevent poor outcomes during this period [127].

Mortality in Children with ESKD

The mortality of children with ESKD has declined over the past few decades in both children treated with dialysis and transplant [2, 4, 136], but the risk of death in children with ESKD remains 30 times higher than that of the general population of the same age [137]. The mortality rate in children with ESKD is about 50–60 deaths per 1000 patient-years (ranging from 15 to 100) [2, 4] depending on age and treatment modality, with mortality risk being highest in those receiving hemodialysis, followed by peritoneal dialysis, and lowest in those with a functioning allograft. The excess mortality attributable to ESKD has been declining over time in children, with the decline being most prominent in young children receiving KRT and with a functioning transplant [138]. Thus, kidney transplantation remains the treatment modality of choice in children, although this is not an option in all nations. The risk of mortality varies substantially across different countries; rates of discontinuation of dialysis and death are high in Africa [139].

The most common cause of death in children treated with KRT are cardiovascular causes in about 35% of patients, followed by infections [104, 113]. Of the cardiovascular causes of death, sudden cardiac death is significantly more common than traditional atherosclerotic disease as the cause of death in those who started KRT as children [113]. Risk factors for death in children treated with KRT include younger age at initiation, female sex, Black race/ethnicity, and late referral to pediatric nephrology care [4, 35, 140–143] .

Conclusion

Although our knowledge of epidemiological data surrounding CKD and ESKD in children has increased substantially over the last few decades, most of the available data rely on national registries and limited data are available on the incidence and prevalence of CKD in children. Standardization of the classification of CKD has helped to streamline research and clinical care for children with CKD. Given the general low prevalence of CKD in children (compared with adults), additional multi-national collaborations are needed to better define the scope of the disease and areas where improvements can be made to prevent the onset of KRT and improve survival in the pediatric population.

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Nutrition, Growth, and Development

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Abstract

Normal growth and development are central goals in the management of children with chronic kidney disease (CKD). Nutritional adequacy plays an influential role in the achievement of these goals. This chapter will review the growth patterns observed in children with CKD as well as the causes of growth impairment and the signs and causes of protein energy wasting (PEW) in pediatric CKD. The methods for assessing growth and nutritional status, along with the limitations of these measures in CKD, will also be reviewed. Management strategies to optimize growth and nutrition among children with CKD, including the use of recombinant growth hormone, will be outlined. Finally, the chapter will conclude with information pertaining to the neurodevelopment of the child with CKD.

Keywords

Chronic kidney disease · Standard deviation score · Body mass index percentile · Final adult height · Pediatric chronic kidney disease · Growth hormone · Dietary reference intakes · Neurocognitive outcomes

Introduction

Normal growth and development are central goals in the management of children with chronic kidney disease (CKD). Nutritional adequacy plays an influential role in the achievement of these goals. This chapter will review the growth patterns observed in children with CKD as well as the causes of growth impairment and the signs and causes of protein energy wasting (PEW) in pediatric CKD. The methods for assessing growth and nutritional status, along with the limitations of these measures in CKD, will also be reviewed. Management strategies to optimize growth and nutrition among children with CKD, including the use of recombinant growth hormone, will be outlined. Finally, the chapter will conclude with information pertaining to the neurodevelopment of the child with CKD.

Normal Growth and Development

Normal Phases of Growth Normal growth can be divided into three phases – infancy, childhood, and puberty. Each phase is primarily influenced by a different factor [1–3]. The *infancy phase* of growth, a continuation of fetal growth, is driven primarily by nutrition. Nutritional intake stimulates insulin and insulin-like growth factors, the major regulators of growth in this phase. Linear growth and weight gain are most rapid in the first 2–3 months of life, showing rapid deceleration thereafter [2]. At 6–12 months of age, the early, nutrition-dominated infancy phase of growth is replaced by the *childhood phase* of growth. Growth hormone is the major driver of growth in the *childhood* phase, during which growth velocity decelerates over time [3]. In the childhood phase, growth rates of ~7–8 cm/year are typical at 3 years of age and of ~6–7 cm/year at 10 years of age. The onset of puberty marks the beginning of the *pubertal phase* of growth, influenced mainly by sex hormones [1–4]. Pubertal growth patterns differ for girls and boys. Girls experience a major increase in growth velocity during the first 2 years of puberty, followed by an additional

2–3 years of slower growth. In contrast, boys show a smaller increase in growth velocity in early puberty, and some may even experience a decline in growth velocity for 12–18 months in early puberty before the growth spurt in mid-puberty. Like in girls, the growth spurt lasts about 2 years and is followed by 2–3 additional years of gradual growth.

Normal Puberty There is substantial variation in the age of onset of puberty in healthy children. Puberty typically begins earlier in girls than in boys, and sexual development is also completed at a younger age in girls than boys. Sexual development is assessed using Tanner staging [5]. For girls, breast and pubic hair development are staged separately. For boys, genital (testicular volume and penile size) and pubic hair development are staged separately. Pubic hair and breast (girls)/genital (boys) staging may be discordant. In boys, testicular volume should be measured using a Prader orchiometer; a testicular volume of 4 ml signals the onset of puberty [6].

Sexual development is considered to be delayed in a girl if there is no breast development by 14 years, if there is more than a 5-year interval between breast development and menarche, or no menarche by 16 years. In boys, the absence of testicular enlargement by 14 years or an interval of more than 5 years between onset and completion of genital development indicates delayed sexual maturation [7].

Growth and Development in Chronic Kidney Disease

Growth disturbance is one of the hallmarks of CKD in childhood [8]. Short stature is common among children with CKD, with younger age of onset, and more advanced CKD associated with larger height deficits. Short stature has long been recognized as a complication of CKD, with 30–50% of individuals with childhood onset CKD being reported as having height deficits as adults [9]. CKD affects each of the phases of growth in a slightly different way.

Growth failure has clinical, social, and mental health implications for children with CKD. There is a significant association between growth failure and increased mortality and morbidity in children with end-stage kidney disease (ESKD). Wong et al. assessed the association between anthropometric measurements and mortality among 1,949 children with ESKD. In this group, the risk of death was 14% higher for every 1 unit increment in height standard deviation score (SDS) [10]. Furth reported that among pediatric patients with ESKD, those with moderate/severe growth failure at the beginning of dialysis had a higher likelihood of hospitalization and death than those with normal stature, after adjustment for relevant covariables [11]. Although growth failure has been reported as a risk factor for adverse clinical outcomes in children with CKD, it has not been independently related with the decline of GFR in the Chronic Kidney Disease in Children (CKiD) cohort study [12]. Nevertheless, growth failure has been associated with a 40% shorter time to deterioration of kidney function after transplantation compared to pediatric kidney transplant recipients with normal stature [13].

There are also significant psychological consequences of growth failure associated with CKD in children. Children with growth failure demonstrate higher rates of poorer school performance and lower self-esteem compared to children with expected height [14]. Al-Uzri and colleagues investigated 483 patients and/or parents recruited in the CKiD study who had completed the Pediatric Quality of Life (QoL) Inventory on at least two visits. They reported that a 1 unit higher height z-score was associated with a seven point higher PedsQL parent-proxy score assessment of physical/social functioning of their children. Interestingly, height is associated with QoL over the entire age spectrum [15]. Among adults who had pediatric onset of CKD, those who achieved a greater final adult height have described a better QoL [16]. Therefore, one of the central goals in treating children with CKD is early intervention to maximize growth and developmental potential.

Disruption of Normal Phases of Growth in CKD During the infancy phase of growth, poor nutritional intake may result in growth restriction. In addition, the childhood phase of growth may be delayed in onset until 2–3 years of age in about 40% of boys and 27% of girls with CKD; 60% of both boys and girls may show a transient resumption of the infancy pattern of growth after starting the childhood phase [4]. Because linear growth is quite slow at the end of the infancy phase and faster at the onset of the childhood phase, delayed onset of the childhood phase or return to the infancy phase will result in overall slower growth than normal.

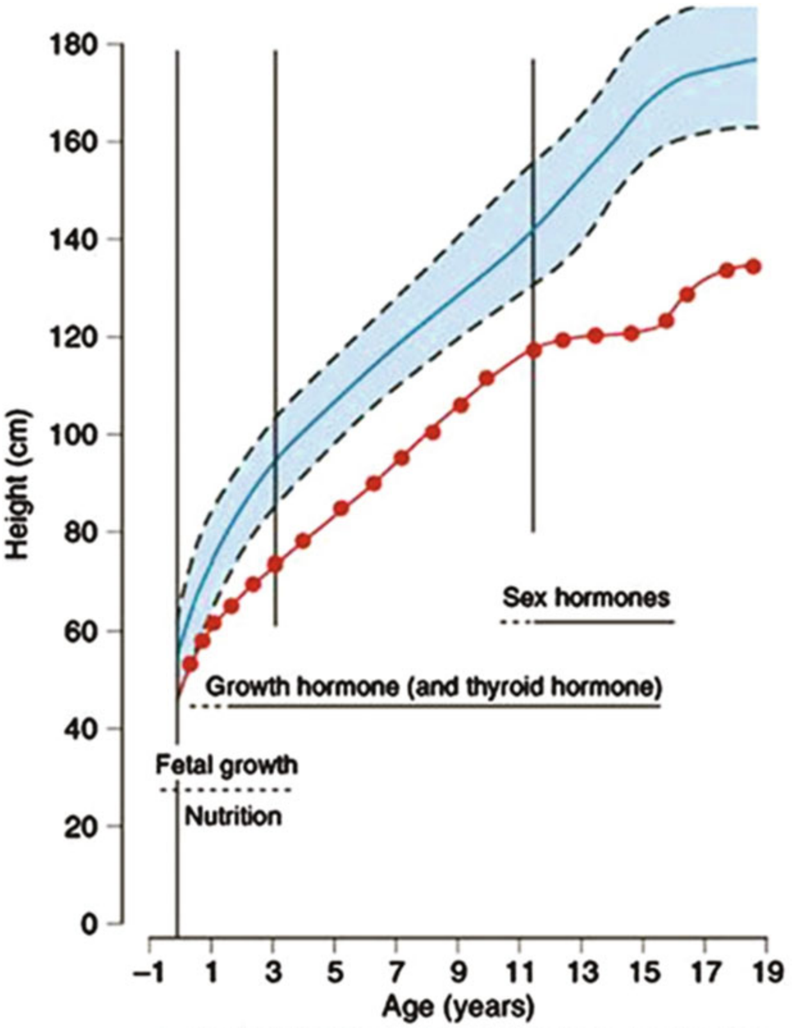
Children with CKD growing in the childhood phase may have normal growth velocity. However, children with a lower glomerular filtration rate (GFR) have significantly lower growth velocity than children with a higher GFR [17]. In addition, the onset of pubertal growth is delayed among children with CKD, and the pubertal growth spurt is shorter [18]. The typical growth pattern for a child with CKD is illustrated in Fig. 1 [19]. It should be noted, however, that most of these observations were made more than 20 years ago and more recent data reveal that even in pre-pubertal patients requiring kidney replacement therapy, pubertal onset may be normal or only slightly delayed [20, 21].

Together, the disturbances in the normal pattern of growth that are characteristic of CKD may lead to severe short stature. Children with CKD from a very young age are particularly severely affected; failure to grow at a normal rate during critical periods when growth is normally rapid is very difficult to correct later.

Pubertal Development in CKD In healthy children, gonadotropin-releasing hormone (GnRH) stimulates the release of luteinizing hormone (LH) in pulsatile nocturnal bursts from the pituitary gland. In CKD, GnRH secretion may be blunted, resulting in a decreased frequency of LH pulses [22]. Although LH levels may be increased in CKD due to decreased renal clearance, pubertal patients receiving maintenance dialysis produce substantially less LH overnight than healthy peers [23]. As a result of these abnormalities, the onset of puberty may be delayed in children with CKD. However, both boys and girls with CKD do eventually progress through the normal stages of puberty and attain full sexual maturation. Because growth and pubertal development are so tightly linked, it is important that pubertal development be carefully monitored in conjunction with growth assessment.

Etiology of Growth Impairment in CKD There are a variety of reasons that children with CKD experience impaired growth. As noted above, poor caloric intake – common among infants with CKD – has the most important effect on growth in the first 1–3 years of life. Anorexia appears to be the primary reason for growth failure in infants and very young children with CKD, and has been observed in infants with glomerular filtration rates as high as 70 ml/min/1.73 m^2 [24]. A variety of factors may cause anorexia in CKD, including disturbances in the appetite-regulating hormones leptin and ghrelin, altered taste, gastroesophageal reflux and frequent emesis, delayed gastric emptying, and elevated levels of cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF)-α [25]. Although some older children may have poor energy intake, this is not common; most older children have normal energy intake relative to body size [8]. Developmental abnormalities and/or psychological factors may also contribute to poor intake. In addition, children with a history of anorexia may have poor oral-motor skills, resulting in very slow eating and low intake. The struggles around eating between parents and anorectic children, and the use of nasogastric tubes, may ultimately result in negative psychological reactions to food and further food refusal [26].

A large proportion of children with CKD have poor urinary concentrating ability and therefore high urine volumes. Such children often feel thirsty and prefer to drink water over milk or other fluids. The volume of water taken may be large enough to diminish their appetite for milk or food. Children with CKD may also experience salt wasting, resulting in chronic volume depletion and impaired growth. Uncorrected chronic metabolic acidosis is an additional factor that may contribute to poor growth [27].



(Reproduced from Mehls O. Schaefer F: Endocrine, metabolic and growth disorders-patterns of growth and maturatin in chronic renal failure-impact of developmental stage. In holiday MA, Barratt TMJ vner ED. editors: *Pediatric nephrology*. Baltimore, 1994, Williams & Wilkins, pp 1260–63.)

Fig. 1 Typical growth pattern in patients with congenital chronic kidney disease is shown in *red*. Loss of statural height predominates in infancy and adolescence, with growth parallel to the normal curves in mid-childhood. The shaded area represents the normal range (3–97%). (Reproduced with permission from Mehls and Schaefer [19])

Disturbances in the growth hormone (GH)–insulin growth factor 1 (IGF-1) axis plays yet another important role in CKD-related growth retardation. The nature of the disturbance depends somewhat on age and involves impaired Janus kinase 2 (JAK2)-signal transducer and activator of transcription (STAT) signal transduction following GH binding to the receptor and increased expression of the growth plate suppressor of cytokine signaling (SOCS2), as described in greater depth later in this chapter [28–31].

Evaluation of Growth, Development, and Nutritional Status in CKD

Because of the high risk for impaired growth, and the unquantified (but at least theoretical) risk for protein energy wasting in children with CKD, it is recommended that their growth and nutritional status be evaluated regularly. The Pediatric Renal Nutrition Taskforce (PRNT) 2020 Clinical Practice Recommendations on the assessment of nutritional status in children with CKD provides guidance on the measures to follow and at what intervals [32]; these recommendations, informed by the 2008 Kidney Disease Outcomes Quality Initiative (KDOQI) recommendations [33], are summarized in Table 1. Recommendations differ for children under 1 year old and for those 1–3 years old versus older children, based on the different factors influencing growth among infants and very young children compared to older children with CKD.

Growth and nutritional status are assessed by measuring recumbent length (in children 24 months of age or younger) or standing height (children >24 months old) and weight. For children under 2–3 years of age, head circumference is also measured. Length should be measured in infants younger than 2 years using a length board, and height should be measured in older children using a wall-mounted stadiometer. Ideally, the same well-trained person should take the measurement at each assessment. Serial measures of length or height can be used to determine height velocity (cm/year). Weight should be measured when the child is determined to be at his/her euvolemic or "dry" weight, or the dry weight should be estimated based on clinical status. Dry weight (in kilograms) is divided by length or height (in meters) squared to calculate the body mass index (BMI). To determine the adequacy of growth and nutritional status, these measures (length or height, BMI, and height velocity) must be compared with reference values for healthy children by plotting them on growth curves and calculating standard deviation scores (SDS). SDS represent the difference, in standard deviation units, between an individual and the mean for healthy children of the same sex and age.

Growth Reference Values The World Health Organization (WHO) growth reference standards [34] provide the recommended reference for growth parameters from birth to 2 years; these standards represent ideal growth, which should be the goal for children with CKD. After 2 years, the WHO growth standards may be used up to age 5 years and the WHO growth reference data thereafter, or country-specific reference curves may be used where available. The reference population used to generate the WHO growth standards was of broad ethnic diversity; only 3% of the variability in length within the WHO population could be attributed to the country of origin [34], suggesting

Table 1 Parameters and frequency of nutritional assessment in children with CKD stages 3b–5D[a]

Measure	Age 0–1 year[b] Minimum interval (weeks)		Age 1–3 year Minimum interval (months)		Age > 3 years Minimum interval (months)	
	CKD 3b-5	CKD 5D	CKD 3b-5	CKD 5D	CKD 3b-5	CKD 5D
Height or length for age centile or SDS	6	2–4	2	1	3	3
Height velocity for age centile or SDS	N/A	N/A	3	2	6	6
Estimated euvolemic weight and weight for age centile or SDS	6	4	2	1	3	3
BMI for height-age centile or SDS	N/A	N/A	2[c]	1[c]	3	3
[c]Weight for length centile or SDS	6	6	2[c]	1[c]	N/A	N/A
Head circumference for age centile or SDS	6	4	2	2	N/A	N/A
Dietary intake	8	8	3	3	6	4

[a] Earlier stages of CKD and other kidney diseases are not addressed in this table, as clinical conditions can vary, and physician discretion is required. Further details are addressed in [33]

[b] Infants and toddlers cannot be categorized with the stage of CKD as there may be spontaneous improvement in kidney function up to 2 years of age. A suggested method to characterize the stage of CKD in this age group is to use the KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease, substituting a GFR > 1 but ≤2 SDS below the mean for moderate reduced GFR (stages 3–4) and severely reduced GFR > 2 SDS below the mean for stage 5 [161]

[c] Weight for length should be used for children <2 years of age or up to 3 years if accurate standing height measurement is not possible

that the growth standards reflect a reasonable expectation for growth regardless of ethnicity. All WHO growth charts can be found at http://www.who.int/childgrowth/en/.

Special growth curves are available for a variety of special populations, including Trisomy 21, Turner syndrome, and premature infants [35, 36]. Diagnosis-specific curves were constructed from fairly small reference populations but provide useful guidance on expected growth in these unique groups. Premature infants should have growth assessed relative to their corrected age rather than chronological age. Corrected age is determined by subtracting the number of weeks early that the child was born from the chronological age. Separate curves are available for low-birth-weight (1,500–2,500 g) and very-low-birth-weight (<1,500 g) infants [37].

Assessing Linear Growth

When assessing the adequacy of growth, it is important to consider the child's height not only relative to the appropriate reference group, but also relative to his or her genetic potential. Although many factors influence growth in addition to genetic factors, a child's adult height potential may be estimated from the sex-adjusted midparental height. Midparental height is calculated from the heights of the biological parents. For girls, 13 cm is subtracted from the father's height and averaged with the mother's height. For boys, 13 cm is added to the mother's height and averaged with the father's height. This average of parental heights is then plotted at age 20 years on the growth chart relevant for the sex of the child; the heights 8.5 cm above and below the calculated midparental height represent two standard deviations around this estimate [33].

Determination of bone age may be helpful in providing an idea as to the remaining growth potential of the long bones. By convention, bone age is evaluated by obtaining a left hand and wrist radiograph. This is compared with the images of "standard" hand/wrist radiographs from children of different ages in the Greulich and Pyle skeletal age atlas [38]. However, this method is imprecise and subject to interobserver variability. Bone age should be recognized as an estimate of skeletal maturity, rather than a precise measure, and should be interpreted in the context of the sexual maturity and overall health.

Height- or Length-for-Age Percentile or SDS The height- or length-for-age SDS is calculated by subtracting the child's height from the mean height for children of the same age and sex and dividing by the standard deviation for children of the same age and sex. A SDS of zero represents an average height; a positive SDS indicates above average height, and a negative SDS indicates below average height. The percentile can either be estimated by plotting on the curve, on which percentile lines are marked, or by calculating it from the SDS.

Height- or Length Velocity-for-Age Percentile or SDS Growth velocity, measured in cm/year, may be calculated to determine if the growth rate is appropriate for age. The simplest way to visualize growth velocity is to plot length or height on the growth curves to determine if the child is crossing percentiles. However, growth velocity can be assessed more precisely using growth velocity reference values. Growth velocity standards are available from the WHO for infants up to 24 months [39], and growth velocity reference values are available for older children [40, 41]; these can be used to calculate growth velocity SDS. A negative height velocity SDS indicates that the child is growing at a lower-than-average rate, resulting in a crossing down of percentiles. A positive height velocity SDS may indicate catch-up growth. Among children over 2 years old, growth velocity and growth velocity SDS can only be accurately estimated over intervals of a minimum of 6 months. However, height may be measured more frequently and plotted on the growth curves to give a general impression of growth adequacy.

Assessing Body Weight

The first step in determining the appropriateness of a child's weight is to estimate the euvolemic weight. This is generally referred to as the "target dry weight," since volume overload is the more common abnormality in CKD; this is discussed further in ▶ Chap. 74, "Assessment of Volume Status in Chronic Kidney Disease." However, some children with CKD may also be at risk for volume depletion due to high urine volumes (e.g., renal dysplasia and cystinosis). Physical findings such as edema and jugular venous distention indicate volume overload. Hypertension is also commonly a sign of fluid overload in patients on maintenance dialysis [42]. In terms of laboratory assessment, hypoalbuminemia or anemia may be a marker for fluid overload. In contrast, thirst, tachycardia, decreased skin turgor, and sunken eyes and/or fontanelle signify volume depletion. In maintenance dialysis patients, response to dialytic fluid removal (hypotension and cramping) may give clues as to the volume status of the patient. Use of blood volume monitoring during a HD session can also be beneficial; this is discussed further in ▶ Chap. 76, "Chronic Hemodialysis in Children." Estimation of target dry weight is difficult, particularly in growing children, in whom weight is expected to increase over time. Most importantly, errors in the estimation of target dry weight may have an important impact on the assessment of the appropriateness of body weight relative to height. For example, an overestimation of target dry weight representing 10% of the body weight will result in a BMI SDS about 0.5–1.0 SD units above what it would be at the true target dry weight.

Estimated Dry Weight and Weight-for-Age and Body Mass Index-for-Age Percentiles or SDS Once the target dry weight has been estimated, it should be plotted on the weight-for-age curves and the SDS calculated. Weight-for-age cannot be interpreted in isolation because it will be low in children who are short for age. Therefore, weight-for-age should be interpreted in the context of the height-for-age percentile or SDS. Appropriateness of weight relative to height is best assessed using weight-for-length percentiles in younger children (<2 years) or BMI in older children.

BMI, calculated by dividing weight (kg) by height (m) squared, provides a means of evaluating the appropriateness of a child's weight relative to height. Because a normal BMI changes with age-related changes in body proportions and body composition, the raw BMI is not meaningful in children. Like other growth parameters, BMI must be expressed as a percentile or SDS relative to age and sex [17] to allow meaningful and consistent interpretation regardless of age. The WHO does provide pediatric BMI reference values from birth [34]; country-specific reference values are also available in some countries.

There are some caveats to the assessment of BMI in children with CKD due to the high prevalence of growth retardation and delayed sexual maturation. If BMI is expressed relative to chronological age in a child with growth and/or maturational delay, this will result in inappropriate underestimation of his/her BMI compared to peers of similar height and developmental age. This problem can be avoided by expressing BMI relative to height-age in children with CKD [43]. Height-age is the age at which the child's actual height would be on the 50th percentile. This approach is currently recommended for children with CKD whose height is below the third percentile for age and who have not yet attained their final adult height [32]. When growth and sexual maturation are complete, BMI should be expressed relative to chronological age.

It is important to recognize that the BMI SDS only provides information on whether the child's weight is above or below the normal healthy range for children of the same height, sex, and age; it does not distinguish fat from lean weight. Nevertheless, BMI SDS remains a useful index of nutritional status. As in healthy children, the BMI percentile or SDS can be used to classify children with CKD as underweight, normal weight, overweight, or obese. The WHO defines underweight or "thinness" as a BMI-for-age less than the third percentile (SDS less than − 2.0), overweight as BMI-for-age greater than the 85th percentile (or more than about 1 SD above the average), and obesity as BMI-for-age above the ~97th percentile (>2 SD above the average) [44]. However, it must be recognized that the risk associated with any of these categories in a CKD population remains controversial. These are distributional cutoffs; no evidence-based definitions link underweight or thinness to poor outcomes in the general population. Although obesity and overweight have been associated with adverse outcomes in otherwise healthy adults, the ability of these categories to predict poor outcomes in the pediatric CKD population is unknown. Large studies of adult hemodialysis patients showed an inverse relationship between BMI and mortality risk. These studies found no clear BMI threshold above which the mortality risk stabilized or began to increase, even as BMI increased above 30 [45, 46]. A smaller study, also of adult hemodialysis patients, found a U-shaped relationship between BMI and mortality risk, with higher mortality risk among those with BMI <17 or >23 compared to those with BMI between 17.0 and 18.9 [47]. A similar U-shaped association has been seen in children with stage 5 CKD: mortality risk was lowest among children with a BMI SDS around +0.5. A 1.0 SD unit difference in BMI SD score in either direction was associated with a 6% higher risk of mortality [10] which represents a fairly small effect. All of these studies can only conclude that there is an association between BMI and outcome; a causal relationship has not been established. Nonetheless, BMI, interpreted in the overall clinical context, can provide useful information to clinicians caring for children with CKD.

Head Circumference-for-Age Percentile or SD Score Like for healthy children ≤2–3 years of age, head circumference should be measured regularly and plotted on the head circumference-for-age curves in children with CKD. The WHO growth standards are the most appropriate reference curves [48]. In the past, infants with CKD were shown to be at risk for poor head growth – reflecting poor brain growth. However, more recent studies, in an era where nutritional support was likely more consistent, have demonstrated mostly normal head growth in infants with CKD [49]. Nevertheless, this measure is still recommended. Head circumference assessment should be coupled with intermittent developmental assessments in this population.

Other Measures

In addition to growth parameters, there are a few other methods that are useful in assessing nutritional status. These include appetite assessment, dietary intake, a nutrition-focused physical exam, and biochemical parameters.

Appetite Assessment A simple assessment of appetite may be a very useful measure of overall well-being. Patients and families can be asked to characterize appetite as very good, good, fair, poor, or very poor at clinical visits. A "very poor" appetite has been associated with a higher risk for hospitalization and poorer quality of life compared with all other categories in children with CKD [50].

Dietary Intake Information on dietary intake allows the treating team to evaluate the adequacy of a patient's intake before significant adverse changes in body composition or growth result. This is particularly important in infants, in whom poor intake is expected. A skilled registered dietician is able to assess dietary intake using a variety of methods. Dietary records allow the dietician to estimate the daily intake of calories, macronutrients (carbohydrates, protein, and fat), vitamins, and minerals. Prospective three-day dietary diaries provide unbiased estimates of energy intake in children under 10 years old. However, underreporting is common among adolescents [51, 52] making 24-h recalls a potentially more suitable option in adolescents. Given the inability of the 24-h recall method to capture the day-to-day variability in intake, it is recommended that three 24-h recalls (including one weekend day and two weekdays) be conducted for a more complete evaluation of intake [32].

Nutrition-Focused Physical Examination The first step in a nutrition-focused physical examination involves a general assessment of volume status, including the presence of edema. This is necessary to allow estimation of dry weight. The physical exam also includes inspection and palpation of areas where there may be muscle and/or fat wasting. Skin, hair, and nails should be examined for signs of micronutrient deficiencies. The nutrition-focused physical exam can be used as part of the subjective global nutritional assessment [53], which includes anthropometric measures, appetite changes, gastrointestinal symptoms, and dietary intake. The subjective global nutritional assessment generates a risk score for malnutrition. Although the subjective global nutritional assessment has limited evidence in the pediatric CKD population [54], it has been validated in a general pediatric population and in adults with CKD [55].

Biochemical Parameters There are few validated biochemical parameters that are useful in assessing nutritional status. The normalized protein catabolic rate (nPCR) is currently the only measure validated for use in pediatric CKD; evidence exists for its validity in adolescents treated with maintenance hemodialysis [56]. The nPCR provides an objective estimate of dietary protein intake; an nPCR of ≥ 1 indicates adequate protein intake in adolescents 13–19 years old on hemodialysis [32]. Sequential measures of nPCR in younger children may be useful in following trends when interpreted in the context of dietary intake, weight gain, and growth.

Serum albumin is frequently suggested as a measure of nutritional status. However, its interpretation is substantially confounded by other factors. Fluid overload will decrease albumin, as will systemic inflammation. Unless all other causes of hypoalbuminemia have been excluded, albumin is not recommended as a method of assessing nutritional status. Urea is also often suggested as a parameter correlated with nutritional status. However, there is very limited evidence of its value as such.

Protein Energy Wasting (PEW)

Adverse changes in nutrition and body composition are prevalent in patients with CKD and are associated with significant morbidity and mortality. Among the many risk factors that affect outcomes in these patients, especially those with advanced CKD, is a state of protein energy wasting (PEW), characterized by anorexia, poor weight gain, and low muscle mass. This is associated with major adverse outcomes, such as an increased frequency of hospitalization [57]. The terms wasting, cachexia, and malnutrition have historically been used interchangeably. However, to be more specific, malnutrition results from the improper intake or absorption of nutrients despite a preserved appetite and manifests as weight loss associated with adaptive metabolic responses. In contrast, wasting syndromes are characterized by maladaptive responses, including anorexia and a high metabolic rate. As a result, wasting syndromes are associated with low serum albumin, weight loss, diminished muscle mass, and normal or increased fat mass. Although inadequate nutritional intake can be partly responsible for the nutritional derangement, recent studies have shown that many other factors, including systemic inflammation, endocrine disturbances, and abnormal neuropeptide signaling, may significantly contribute to a wasting syndrome in the setting of CKD [57]. The concept of protein energy wasting was defined by the International Society of Renal Nutrition and Metabolism (ISRNM) as a state of nutritional and metabolic derangements in patients with CKD characterized by the simultaneous loss of systematic body protein and energy stores, leading to the loss of muscle and fat mass and ultimately cachexia [58]. Thus, PEW is a pathological state in which there is a persistent decline or wasting of both protein deposits and energy reserves. A recent study made an important contribution to the definition of PEW in children with CKD by including growth/growth velocity as a diagnostic parameter [59]. The addition of this indicator to existing adult criteria improved the prediction of hospitalizations, and it is thus important that growth be considered when assessing children at risk for PEW. Abraham et al. provided the criteria for pediatric PEW (Table 2) in the analysis from the CKiD study. Among the 528 children analyzed in this study [median age 12 years; median glomerular filtration rate (GFR) 45 ml/min/1.73 m^2], PEW prevalence ranged from 7–20% depending on the definition used. There was also a higher, but not statistically significant, prevalence of PEW [odds ratio (OR) = 1.5] in CKD stages 4 and 5. Interestingly, low muscle mass was reported to be highly prevalent and trended toward a higher frequency

Table 2 Diagnostic criteria for PEW in children with CKD [59]

Biochemical – Serum total cholesterol (TC) $<$ 100 mg/100 ml; serum albumin $<$3.8 g/100 ml, serum transferrin $<$140 mg/dl; CRP $>$3 mg/L
BMI for height/age and sex $<$5th percentile at diagnosis of CKD or a decrease in BMI $\geq$ 10% between the first and second annual visits
Mid-upper-arm circumference (MUAC) for height/age and sex $<$5th percentile or a decrease in MUAC $\geq$10% between annual visits
Decreased appetite
Poor growth – defined as either short stature [a height/age and sex percentile $<$3% or poor growth velocity (decrease in height for age and sex percentile of $\geq$10% between annual visits)

with lower GFR [59]. Foster et al. also evaluated whole/regional lean and fat mass in 143 children with CKD and 958 healthy individuals. They reported that leg lean mass Z-scores were similar in CKD stages 2–3, but were lower in stages 4–5 compared with controls [60].

Decreased physical activity is likely to play a major role in the pathophysiology of PEW. There has been increasing recognition of the benefits of regular physical activity for CKD patients, which improves cardiac function, decreases cardiovascular risk factors, enhances muscular strength, reduces the number of atrophic muscle fibers, and increases exercise capacity. A recent systematic review confirmed that progressive resistance training induces skeletal muscle hypertrophy, increases muscular strength, and improves health-related quality of life of CKD patients [61]. Indeed, exercise is also important in the management of children with ESKD, especially those with poor nutrition and growth failure [62]. Although its long-term effect on clinically relevant outcomes remains to be determined, resistance exercise training is safe, effective, and costless, and should be encouraged as a potential countermeasure for PEW [61].

As noted previously, perturbations in appetite-regulating hormones such as leptin and ghrelin may contribute to anorexia in CKD [63]. As the kidney degrades leptin, CKD is independently associated with a peripheral increase in circulating leptin concentrations that are strongly and inversely correlated with GFR in children [63]. Experimental anorexia and cachexia of CKD can be ameliorated by blockade of leptin signaling through the hypothalamic melanocortin-4 receptor [64]. Human data on leptin and CKD–PEW are inconclusive. With body fat mass loss, serum leptin will primarily reflect these fat stores, thus obscuring any links to appetite [63]. Cheung et al. tested whether treatment with a pegylated leptin receptor antagonist (PLA) attenuates cachexia in mice with CKD. PLA stimulated appetite and weight gain, improved lean mass and muscle function, reduced energy expenditure, and normalized the expression of proinflammatory muscle cytokines such as IL-1α, −1β, and − 6 and TNF-α. These results suggest that leptin antagonism may represent a viable therapeutic strategy for cachexia in CKD [65]. Increased total ghrelin levels in CKD are primarily due to the decreased degradation of ghrelin in the kidney. Recent studies have suggested that elevated des-acyl ghrelin levels could be involved in the anorexia of CKD patients [66]. Short-term ghrelin administration can improve appetite, but long-term trials of ghrelin in CKD patients have not been reported [67].

Increased energy expenditure despite caloric insufficiency is an important feature of cachexia often observed in CKD. Ikizler et al. carefully studied adult ESKD patients on maintenance hemodialysis and showed a higher resting energy expenditure (REE) which was further increased during hemodialysis [68]. Wang et al. studied a sizable population of 251 adult peritoneal dialysis patients, who were classified by REE tertiles. They showed that patients in the upper and middle tertiles of REE showed a significantly higher risk of all-cause mortality compared to those patients in the lower REE tertile [69].

Adipose tissue and muscle are both involved in energy metabolism. Uncoupling protein (UCP)-1 is important for thermogenesis, while UCP-2 and UCP-3 regulate mitochondrial energy metabolism. Increased UCP expression stimulates

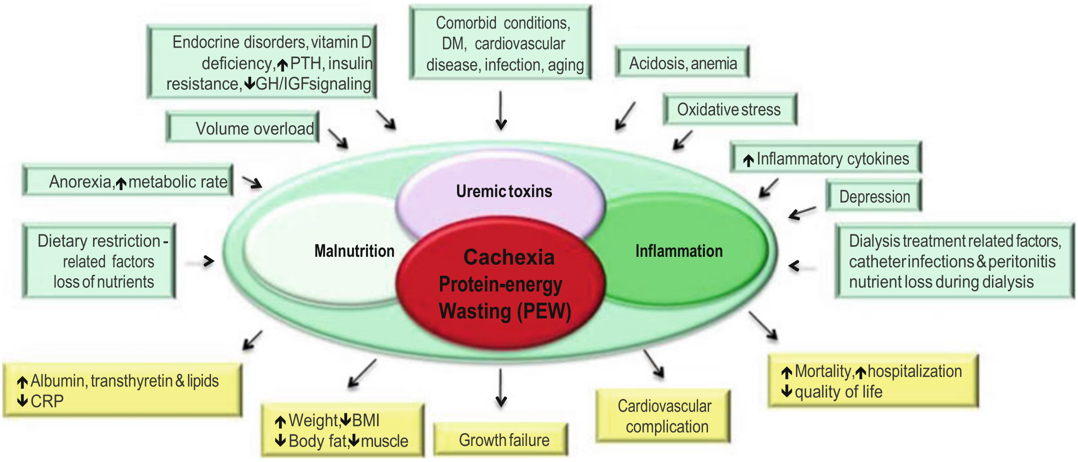

Endocrine disorders, vitamin D deficiency, ↑PTH, insulin resistance, ↓GH/IGF signaling
Comorbid conditions, DM, cardiovascular disease, infection, aging
Acidosis, anemia
Oxidative stress
Volume overload
↑Inflammatory cytokines
Anorexia, ↑metabolic rate
Depression
Uremic toxins
Malnutrition
Cachexia Protein-energy Wasting (PEW)
Inflammation
Dietary restriction - related factors loss of nutrients
Dialysis treatment related factors, catheter infections & peritonitis nutrient loss during dialysis
↑Albumin, transthyretin & lipids
↓CRP
↑Weight, ↓BMI
↓Body fat, ↓muscle
Growth failure
Cardiovascular complication
↑Mortality, ↑hospitalization
↓quality of life

Fig. 2 Cachexia/PEW in CKD. Manifestations and pathogenetic factors of cachexia/PEW in CKD [74]

thermogenesis while inhibiting ATP synthesis. Cheung et al. showed that adipose tissue's UCP content was increased while ATP content was significantly decreased in CKD. This phenomenon is described as adipose tissue browning and provides an important mechanism for hypermetabolism in CKD [70].

Various observational studies and prospective randomized trials have provided convincing evidence that recombinant human growth hormone (rhGH) can improve nutritional biomarkers and LBM in maintenance dialysis patients. A large multicenter randomized controlled trial was designed to investigate whether rhGH reduces mortality and morbidity and improves overall health in adult patients on dialysis with low serum albumin. Although the study was terminated early because of low recruitment, rhGH therapy improved some cardiovascular risk factors, but did not reduce mortality or ameliorate nutritional indexes [71]. Recent findings in rodent studies have also suggested that increased serum levels of PTH [72] and/or vitamin D deficiency [72] may be significant factors for CKD cachexia. Cheung et al. demonstrated improvement in muscle wasting and cachexia by vitamin D repletion, an intervention that was accompanied by normalization of serum 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 concentrations and attenuation but not normalization of the serum PTH concentrations in CKD mice. Based on these results, it was postulated that the impact of secondary hyperparathyroidism may be less significant than the influence of vitamin D insufficiency in CKD-associated cachexia and muscle wasting [73]. However, these preliminary preclinical studies ideally need to be confirmed by randomized trials in children with CKD. The clinical manifestations and factors contributing to PEW in children with CKD are summarized in Fig. 2 [74].

Nutritional Requirements

The nutritional requirements for children with CKD and those undergoing maintenance dialysis are generally based on the published recommended dietary allowances (RDA) for healthy children, and the basis for the RDA values varies for different nutrients. Currently, the RDAs for a number of nutrients have been replaced by dietary reference intakes (DRIs) that are comprised of a set of four reference values: *estimated average requirement* (EAR), *recommended dietary allowance* (also popularly know as recommended daily allowance) (RDA), *adequate intake* (AI), *and tolerable upper intake level* (UL) [75]. Because multiple national and international bodies have published slightly different recommended values and have used terms that are not directly comparable, the Pediatric Renal Nutrition Taskforce (PRNT) decided to take a more pragmatic approach and has recently introduced the novel term suggested dietary intake (SDI), which comprises a range of values [76].

Energy Requirements

Energy requirements are determined by lean body mass, are modified by growth, current nutritional status, and physical activity, and are influenced by genetics, ethnicity, and environment. The optimal energy intake from food should maintain normal body mass, promote growth and development, and support the level of physical activity performed by the subject. Studies that have investigated the optimal requirements for energy in children with CKD found no difference in the resting energy expenditure (REE) (also known as basal metabolic rate (BMR)) between these children and healthy children, after adjusting for lean body mass [77]. Most studies in children with CKD have reported that a dietary energy intake of around 100% of the estimated energy requirement (EER) for chronological age resulted in acceptable growth. The energy intake can be further adjusted based upon response in terms of the rate of weight gain/loss that is individually adjusted for the physical activity level (PAL) and BMI [75, 78].

Energy requirements for patients treated with maintenance HD or PD are similar to those of pre-dialysis patients. In children receiving maintenance PD, variable glucose absorption takes place from dialysis fluid depending on the PD modality, dialysate glucose concentration, and peritoneal membrane solute transport capacity [79]. In a study of 31 children older than 3 years on ambulatory PD therapy, the mean energy intake derived from peritoneal glucose absorption was 9 kcal/kg/day [80]. Since many children who receive chronic PD are underweight, the prescribed energy intake in them should exclude the estimated calorie absorption from the dialysate, while in the setting of obesity, the caloric contribution from dialysate should be taken into account when estimating energy requirements.

Historically, a number of studies have shown that pediatric patients with CKD exhibit inadequate dietary energy intake in association with worsening kidney function [8]. In a prospective study of growth failure in children with CKD, caloric intakes <80% of the RDA for age were present in more than one-half of food records obtained [81]. While an inadequate voluntary energy intake has been clearly demonstrated in infants with CKD [82], energy intakes for older children are generally normal relative to their body size [81]. In contrast to prior publications, the recent assessment of dietary intake in children with CKD from the CKiD study revealed that the median energy consumption levels were higher than recommended values in all age groups. While there was a decreased energy consumption with the fall in GFR ($p = 0.003$), younger children were more likely to exceed the recommended caloric intake than their older counterparts ($p < 0.001$) [83].

Maximizing caloric intake has been noted to be particularly effective in improving height velocity in infants with CKD or receiving dialysis [82, 84]. As children older than 2 years of age with CKD do not generally experience catch-up growth, the provision of adequate energy intake early in life is crucial.

While worldwide, undernutrition in children with CKD remains a concern, recent data from the International Pediatric Peritoneal Dialysis Network (IPPN) reported a 19.7% prevalence of overweight/obesity (compared with 8.9% underweight) in children at the start of maintenance PD [85]. Multivariate analysis revealed that nasogastric/gastric tube feeding was an independent predictor of overweight/obesity [86]. Accordingly, prevention and treatment of obesity in patients with CKD is important. Energy requirements for overweight or obese children are lower than normal and can be estimated by using equations specific for children heavier than a healthy weight [75].

Protein Requirements

The usual dietary protein intake (DPI) requirement is based on nitrogen losses in the urine, stool, hair, and nails, in addition to the extra protein required for growth. It is important that the required protein intake be accompanied by adequate sources of nonprotein energy (i.e., carbohydrates and fats) as inadequate caloric intake results in inefficient use of dietary protein as a calorie source, with a resultant increased generation of urea. Thus, ensuring that caloric needs are met is an important step in assessing protein requirements. It is advised that at least 50% of the total protein intake consists of protein of high biologic value such as that derived from milk, eggs, meat, fish, and poultry. However, more recently, it has been argued that plant-based proteins are not only nutritionally adequate, but have other beneficial effects that may favor their use in CKD patients [87].

The 2009 KDOQI pediatric nutrition guidelines recommended maintaining the DPI at 100–140% of the DRI for ideal body weight in children with CKD stage 3 and 100–120% of the DRI in children with CKD stages 4–5 (Table 3) [33]. The recently published PRNT clinical practice recommendations suggest that the target protein intake in children with CKD 2–5D should be at the upper end of the recommended intake to promote optimal growth, and the DPI should not be reduced below the lowest end of the recommended range which is considered the minimum safe amount [76].

As there is a nearly linear relationship between protein and phosphate intake, the consumption of a high-protein diet in patients with CKD is frequently associated with the development of hyperphosphatemia [88]; this is discussed further in ▶ Chap. 72, "Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD)." Conversely, low-protein diets decrease the development of hyperphosphatemia, metabolic acidosis, hyperkalemia, and other electrolyte disorders. Two randomized controlled trials comparing low-protein versus normal protein diets in children with CKD [89, 90] showed that a modest reduction in protein intake, with maintenance of energy intake greater than 80% RDA in both groups, did not adversely affect growth, serum albumin, or the rate of CKD progression over 2–3 years. Hence, although there is no evidence for a nephroprotective effect of dietary protein restriction, these studies did provide evidence that dietary protein intake can be safely restricted to 0.8–1.1 g/kg/d in children older than 2 years with CKD [90].

While the spontaneous DPI is reduced in association with CKD progression in a manner similar to that of energy intake, the DPI typically still remains far in excess of the average requirements, ranging from 150–200% of the RDA [81, 90]. The recent assessment of dietary intake from the CKiD study revealed that the median protein intake exceeded the recommended levels in all age groups [83].

There are situations in which the dietary protein requirements may be increased relative to the general recommendations. In patients with ongoing proteinuria or during recovery from intercurrent illness, the recommended intake may be adjusted to height-age instead of chronological age if evidence of protein deficiency exists or persists. Modification of protein recommendations may also be necessary in obese children. Obese individuals have a greater percentage of

Table 3 Recommended dietary protein intake in children with CKD stages 3–5 and 5D [33]

Age	DRI (g/kg/d)	Recommended for CKD stage 3(g/kg/d) (100–140% DRI)	Recommended for CKD stages 4–5 (g/kg/d) (100–120% DRI)	Recommended for HD (g/kg/d)[a]	Recommended for PD (g/kg/d)[b]
0–6 months	1.5	1.5–2.1	1.5–1.8	1.6	1.8
7–12 months	1.2	1.2–1.7	1.2–1.5	1.3	1.5
1–3 years	1.05	1.05–1.5	1.05–1.25	1.15	1.3
4–13 years	0.95	0.95–1.35	0.95–1.15	1.05	1.1
14–18 years	0.85	0.85–1.2	0.85–1.05	0.95	1.0

[a] DRI + 0.1 g/kg/d to compensate for dialysis losses

[b] DRI + 0.15–0.3 g/kg/d depending on patient age to compensate for peritoneal losses

body fat, which is much less metabolically active than lean body mass. Therefore, it is believed that basing protein (and energy) requirements of obese individuals on actual weight may overestimate requirements. Conversely, the use of ideal body weight as a reference for an obese person does not take into account the increase in body protein needed for structural support of extra fat tissue. Therefore, a common practice is to estimate protein requirements of obese individuals based on an "adjusted" weight (i.e., adjusted weight = ideal weight for height + 25% × [actual weight − ideal weight], where 25% represents the percentage of body fat tissue that is metabolically active) rather than their actual body weight [91].

Additionally, the optimal protein intake for pediatric patients on maintenance dialysis has not yet been well defined. Although dialyzed children require larger amounts of protein per unit of body weight compared to adults in order to grow in size and lean body mass, this demand is fully accounted for by the age-adjusted pediatric DRI. Hence, the only additional dietary protein requirement is the replacement of dialytic nitrogen losses. In those on long-term PD therapy, daily peritoneal protein losses decrease with age across childhood from an average of 0.28 g/kg in the first year of life to less than 0.1 g/kg in adolescents [92]. Peritoneal amino acid losses add approximately one-third to the nitrogen lost with protein, resulting in a total additional dietary protein requirement of 0.15–0.35 g/kg, depending on patient age (Table 3).

Patients with high peritoneal membrane transport characteristics tend to have low serum albumin levels, likely due to increased peritoneal protein losses; these patients may have slightly greater protein requirements.

Amino acid and protein losses during HD vary according to dialyzer membrane characteristics and reuse. Whereas losses have not been quantified in children, an average of 8–10 g of amino acids and less than 1–3 g of protein are lost per HD session in adults [93]. On the basis of three HD sessions per week for a 70 kg adult, this equates to 0.08 g/kg/day. Assuming that dialytic amino acid losses are linearly related to urea kinetics, children can be expected to have similar or slightly higher amino acid losses than adults and an added DPI of 0.1 g/kg/d should compensate for pediatric HD losses.

Finally, there is some concern that a high DPI may prove harmful when consumed by dialyzed children. In a DXA study of body composition in 20 children on long-term PD therapy with a mean DPI of 144% RDA, protein intake inversely correlated with bone mineral density, bone mineral content, fat-free mass, and plasma bicarbonate level, suggesting that a high protein intake may cause tissue catabolism and bone loss by worsening metabolic acidosis [94]. However, the most convincing argument for limiting DPI in dialyzed children is derived from the solid evidence supporting the key etiologic role that dietary phosphorus load has in the pathogenesis of dialysis-associated calcifying arteriopathy. As noted previously, there is a nearly linear relationship between protein and phosphorus intake, which results in the frequent association between high dietary protein and hyperphosphatemia [95]. Hence, it appears appropriate to limit dietary protein intake in children on dialysis to the safe levels known to ensure adequate growth and nutrition in healthy children.

Lipid Requirements

Dyslipidemia is a frequently recognized complication of CKD in children [96], occurs relatively early in the course of CKD (i.e., stage 3 CKD), and increases in prevalence with decreasing kidney function [96]. The CKiD study reported the presence of dyslipidemia in 44% of 250 children with mild to moderate CKD, with the most common abnormality being hypertriglyceridemia in 75% [97]. Declining GFR and worsening proteinuria are independently associated with worsening dyslipidemia [96]. The dyslipidemia pattern seen in children with CKD has been labeled "atherogenic" and in addition to hypertriglyceridemia, there are high levels of VLDL and intermediate-density lipoproteins (IDLs), low levels of HDL particles, and normal or modestly increased levels of total and low-density lipoprotein (LDL) cholesterol [98].

The 2013 KDIGO CPG for lipid management in CKD recommends obtaining a lipid profile (total, LDL, and HDL cholesterol, and triglycerides) in all children with newly diagnosed CKD (including those receiving dialysis or with a kidney transplant), and with annual follow-up measurements [99], while the optimal management of dyslipidemia in children with CKD is not clearly defined. Treatment of malnutrition related to impaired kidney function is essential and should supersede any potential rise in lipid levels that might result from it. On the contrary, prevention and treatment of obesity in patients with CKD is an important strategy to reduce the risk of hyperlipidemia. Correction of metabolic acidosis, vitamin D therapy, and correction of anemia with erythropoietin each also seem to have some normalizing effect on dyslipidemia in children with CKD [100–102]. The KDOQI dyslipidemia guidelines' recommendations [103], endorsed by the KDOQI cardiovascular guidelines [104], state that dietary and lifestyle recommendations made for adults are also appropriate for post-pubertal children and adolescents with CKD. Therapy with statins or statin/ezetimibe combination is not recommended for children with CKD less than 18 years of age [99].

The expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents recommends that in children with hypercholesterolemia, less than 25–30% of calories should come from dietary fat, of which $\leq$7% should be from saturated fatty acids; the daily cholesterol intake should be $<$200 mg [105]. For serum triglyceride $>$150 mg/dL, therapeutic lifestyle changes (TLC) are recommended along with a low-fat diet and a low intake of simple carbohydrates [99]. The child should be encouraged to ingest complex carbohydrates in lieu of simple sugars and concentrated sweets and to use unsaturated fats such as oils and margarines from corn, safflower, and soy.

High intakes of n-3 polyunsaturated fatty acids (omega-3 fatty acids [n-3 FA], docosahexanoic acid [DHA], and eicosapentanoic acid [106]) have been associated with decreasing triglyceride levels and a decreased risk of heart disease [107]. Therefore, EPA and DHA, found almost exclusively in fish and marine sources, should be provided in the diet; the best sources are fatty fish (e.g., tuna, mackerel, trout, salmon, herring, sardines, and anchovies). Although n-3 FAs have been found to be extremely safe by both Health Canada and the US Food and Drug Administration, there is insufficient evidence to recommend routine use of n-3 FAs to treat hypertriglyceridemia in children with CKD.

Dietary fiber, particularly naturally occurring viscous fiber, reduces total and LDL cholesterol levels and high intakes have been associated with reduced rates of CVD. The AI for total fiber is based on daily caloric intake and is 14 g/1000 kcal/d for all children 1 year and older. Dietary fiber is found in most fruits, vegetables, legumes, and whole grains, which are foods restricted in low-potassium and low-phosphorus diets; therefore, meeting normal daily fiber recommendations is challenging for children with CKD. Tasteless mineral- and electrolyte-free powdered forms of fiber (e.g., Unifiber® and Benefiber®) are available to add to meals or drinks if children are unable to meet their recommended fiber intake by diet. High-fiber diets require additional fluid intake, which may not be possible for oliguric or anuric patients with a strict fluid restriction.

Bone Mineral Metabolism

Optimal control of bone and mineral homeostasis is essential, not only for the prevention of debilitating skeletal complications and the achievement of adequate growth, but also for the prevention of vascular calcification and cardiovascular disease. Complications of mineral bone disease (MBD) are, however, common and contribute to the high morbidity and mortality seen in children with CKD. The provision of adequate but not excessive amounts of calcium and phosphorus is thus an important part of CKD-MBD management; this

Table 4 Recommended SDI (suggested dietary intake) for calcium and phosphate in children with CKD stages 2–5D [108]

Age	SDI calcium (mg/day)	SDI phosphorus (mg/day)
0–< 4 months	220	120
4–< 12 months	330–540	275–420
1–3 years	450–700	250–500
4–10 years	700–1000	440–800
11–17 years	900–1300	640–1250

is discussed further in ▶ Chap. 72, "Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD)." The SDI of calcium and phosphorus is provided in Table 4.

Calcium Adequate dietary calcium intake during childhood is necessary for skeletal development and acquisition of optimal peak bone mass. The current recommendation is that patients with CKD should achieve a calcium intake of 100% of the DRI, just as their healthy peers. The main dietary sources of calcium for children are milk, milk products, breast milk, and manufactured infant formulas. Infants and young children usually meet the DRI for calcium with the consumption of adequate volumes of breast milk/infant formula. All standard and most specialized infant milk formulas have a higher concentration of calcium compared to human milk, but lower than cow's milk or the formulas intended for older children. Formulas designed for infants must comply with set compositional criteria, providing a minimum amount of 50–60 mg of calcium per 100 calories and a Ca:P ratio between 1.1 and 2.0. The calcium content of reduced-fat dairy products is comparable to whole milk-derived products. The "milk and milk products" food group (milk, cheese, yogurt, cream, and ice cream) contribute 44–70% of dietary calcium intake, being highest in the younger age groups. "Cereal and cereal products" (grains made into pasta, rice, breads, breakfast cereals, and biscuits) contribute 16–28% of average daily calcium intake. The variations in foods eaten in different countries or cultures may alter the relative calcium intake from specific food groups [108].

Most problematic is the fact that the largest source of dietary calcium for most persons are dairy products which are also rich in phosphorus; in turn, phosphorus restriction in the patient with CKD universally leads to a decreased calcium intake. In these situations, calcium supplementation may be required as low phosphorus, high calcium containing foods such as collards, dandelion greens, kale, rhubarb, and spinach usually do not make up a substantial part of a child's diet. Several products fortified with calcium such as fruit juices and breakfast foods are commercially available, and limited studies have suggested that the bioavailability of calcium from these products is at least comparable to that of milk. Calcium can also be supplemented in medicinal forms such as carbonate (40% elemental calcium), acetate (25% elemental calcium), and gluconate (9% elemental calcium) salts of calcium that are used as phosphate binders. When used for calcium supplementation alone, ingesting these products between meals maximizes calcium absorption. Chloride and citrate salts of calcium should be avoided as the former may lead to acidosis in patients with CKD and the latter may enhance aluminum absorption.

Excessive calcium intake in conjunction with activated vitamin D analogs can lead to (i) hypercalcemia, (ii) adynamic bone disease, and (iii) systemic calcification. Therefore, the total calcium intake from diet and medications (including phosphate binders) should be within the DRI, and no more than twice the DRI, unless there are exceptional circumstances such as in infants with mineral-depleted bones. Accordingly, the KDOQI guidelines as well as the PRNT recommendations state that the combined elemental calcium intake from nutritional sources and phosphate binders should not exceed two times the DRI for age, except for ages 9–18 years (both genders) where two times the DRI (2600 mg) exceeds the tolerable upper intake level (UL) of 2500 mg [33, 108]. The serum level of total corrected calcium should be maintained within the normal range (8.8–9.5 mg/dL), preferably toward the lower end and definitely not more than 10.2 mg/dL. The calcium balance in patients undergoing maintenance dialysis is also affected by the dialysate calcium concentration; this is discussed further in ▶ Chap. 72, “Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD).”

Phosphorus In an effort to prevent/control CKD-MBD and CVD, serum phosphorus concentrations above the normal reference range for age should be avoided in patients with advanced CKD. However, even during the earlier stages of CKD when the serum phosphorus levels are typically within the normal range, the dietary phosphorus load is an important determinant of the severity of hyperparathyroidism. Dietary phosphorus restriction decreases PTH levels and increases 1,25(OH)$_2$D, whereas a dietary phosphorus intake approximately twice the DRI for age aggravates hyperparathyroidism despite little or no change in the serum phosphorus level (the result of elevated FGF-23 levels and enhanced phosphorus excretion). It is important to note that higher physiological serum concentrations of calcium and phosphorus that are observed in healthy infants and young children presumably reflect the increased requirements for these minerals by the rapidly growing skeleton. Rickets due to phosphorus deficiency can occur in preterm infants whose diet provides insufficient quantities of phosphorus, as well as in infants and children with hypophosphatemia due to inherited disorders of renal phosphate transport. Hence, when dietary phosphorus is restricted to control hyperphosphatemia and SHPT, subnormal serum phosphorus values are equally important to avoid; this is discussed further in ▶ Chap. 72, “Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD).”

Data from the USA for all age groups indicate that the highest contributors to phosphorus intake are milk, meat, and grains [109]. The highest percentage of dietary phosphorus comes from “milk and milk products” particularly in the younger age groups. Breast milk is relatively low in phosphate, having a concentration about one-half of most standard whey-based formulas and one-third of casein-based infant formulas. Phosphate additives, such as phosphoric acid and sodium phosphate, are used by the food industry to improve the texture, taste, appearance, and shelf-life of many processed foods and their presence, but not quantity, is usually indicated on the ingredient list [110]. In addition, inorganic phosphates, which are readily absorbed, are added to several medications, such as antacids and many antihypertensive medications, as an excipient that aids in dispersion of the active drug ingredient once ingested [111]. The phosphorus load from medications can make a meaningful contribution to the daily phosphorus intake, and thus should be reviewed carefully by the health care team.

The absorption of phosphorus from food depends on whether it is in an organic or inorganic form. Meats, fish, dairy, vegetables, grains, and nuts contain organic carbon-bound phosphorus, with a bioavailability between 30% and 70%. Plant-based organic phosphorus (including seeds and legumes) is stored in the form of phytate or phytic acid, which cannot be broken down by humans, reducing the bioavailability to 30–40%.

Current recommendations from KDOQI suggest that in children with CKD whose serum PTH concentration exceeds the target range but whose serum phosphorus concentration remains normal, the dietary phosphorus intake should be restricted to 100% of the DRI; in contrast, the intake should be restricted to 80% of the DRI (which usually corresponds to the lower limit of the SDI) when the serum phosphorus concentration exceeds the normal reference range for age [33, 78, 108].

Despite the need to restrict dietary phosphorus, most clinicians recognize that an overly restrictive dietary phosphorus intake can be ill advised as it may lead to an inadvertent poor dietary protein intake [112]. In addition, extremely low phosphorus diets are typically unpalatable. Young infants are characteristically managed with a low phosphorus containing milk formula such as Similac PM 60/40® (Abbott Nutrition), Renastart®, (Vitaflo Nutrition), Renalcal® (Nestle Health Sciences), or by pretreatment of breast milk, infant formula, and cow’s milk with sevelamer carbonate (Renvela®; Sanofi US) which can effectively reduce the phosphorus content in the supernatant by 80–90% [113]. Whereas some infants may require phosphorus supplementation in the form of sodium phosphate (Neutra-Phos) because of their higher physiological needs, most other patients with CKD require oral intestinal phosphate binders to control hyperphosphatemia. Although food labels rarely state the phosphorus content [110], chocolates, nuts, dried beans, and cola soft drinks are rich in phosphorus and should be avoided; nondairy creamers and certain frozen nondairy desserts may be used in place of milk and ice cream.

Vitamin D Vitamin D plays an important role in the management of CKD-MBD and its deficiency is a risk factor for SHPT and decreased serum 1,25 $(OH)_2D$ levels. The CKiD study observed vitamin D deficiency (<20 ng/mL) in 28% of 506 children at enrollment. Those with 25-hydroxyvitamin D [25(OH)D] deficiency were more likely to be of non-White race, older with higher BMI z-scores, lower milk intake, not receiving a vitamin D supplement, had more proteinuria, and had higher FGF-23 levels [114]. While the total 25(OH)D and vitamin D–binding protein (VDBP) levels are lower in healthy Black adults and Black children when compared to White individuals, the bioavailable 25(OH)D levels are not different [115]. Current guidelines suggest checking serum 25(OH)D levels once every 6–12 months in children with CKD stages 2–5 [33, 116]. If the serum level of 25(OH)D is <30 ng/ml, supplementation with vitamin D_2 (ergocalciferol) or vitamin D_3 (cholecalciferol) is suggested, with the specific dosing regimen dependent on the patient's age and the severity of the deficiency; it is important to note that intermittent megadose regimens are not recommended (Table 5). Further details on vitamin D deficiency in CKD and supplementation are discussed in ▶ Chap. 72, "Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD)."

During supplementation, vitamin D intake from milk formula and fortified foods should also be taken into account. Serum calcium, phosphorus, and urinary calcium should be monitored during the high dose "intensive replacement phase" to evaluate for the development of vitamin D toxicity. Once patients are replete with vitamin D, supplemental vitamin D should be continued and 25(OH)D levels checked every 6–12 months [33, 116].

Acid-Base and Electrolytes

Acid-Base Status Infants and children normally have a relatively larger endogenous hydrogen ion load (2–3 mEq/kg) than do adults (1 mEq/kg); in turn, metabolic acidosis is a common manifestation of CKD in children and an important negative influence on growth. Furthermore, studies performed in adults and children have shown that

Table 5 Suggested treatment for Vitamin D supplementation in children with CKD and on dialysis [116]

Age	Serum 25 (OH)D level (ng/mL)[a]	Vitamin D status	Vitamin D supplementation Dose (IU/day)	Monitoring
Intensive replacement phase				
<1 year	–		600[b]	Serum and urinary calcium levels 1–3 monthly based on CKD stage 25(OH)D levels: after 3 months
>1 year[c]	<5	Severe deficiency	8000	
	5–20	Deficiency	4000	
	21–30	Insufficiency	2000	
Maintenance phase				
<1 year	>30[d]		400	25(OH)D levels: 6–12 monthly
>1 year[c]	>30[d]		1000–1200	

[a] To convert ng/mL to nmol/L multiply by 2.5

[b] In infants <1 year, a fixed dose is recommended irrespective of the level of 25(OH)D

[c] Consider adjusting dose by body size (weight or body surface area)

[d] If levels remain <30 ng/mL, then give doses per the "Intensive replacement" schedule for a further course of intensive replacement and recheck levels

chronic acidosis is associated with increased oxidation of branched-chain amino acids, increased protein degradation, decreased albumin synthesis, and a higher risk of CKD progression [117, 118]. Persistent acidosis also has detrimental effects on bone because it alters the normal accretion of hydroxyapatite into bone matrix and causes bone demineralization as bone buffers are increasingly used for neutralizing the excess acid load. Thus, it is recommended that the serum bicarbonate level should be maintained at or above 22 mEq/L in children with CKD by supplementing with oral bicarbonate as needed [33].

Sodium Sodium requirements in children with CKD are dependent on the underlying kidney disease and the degree of impaired kidney function. Children who have CKD as a result of obstructive uropathy or renal dysplasia are most often polyuric and may experience substantial urinary sodium losses despite advanced degrees of CKD. Sodium depletion adversely affects growth and nitrogen retention [119], and its intake supports normal expansion of the extracellular fluid volume needed for muscle development and mineralization of bone. It is important to note that normal serum sodium levels do not rule out sodium depletion and the need for supplementation. Sodium supplementation can be given as chloride or bicarbonate, depending upon the patient's acid-base status.

In contrast, children with CKD resulting from a primary glomerular disease, or those who are oliguric or anuric, typically require a sodium and fluid restriction to minimize fluid gain, edema formation, and hypertension. The sodium intake for children older than 2 years should be restricted to <1,500 mg (65 mmol) [106], which corresponds to sodium intake of 1–2 mmol/kg/day for those younger than 2 years. These patients should be advised to avoid processed foods and snacks from fast-food restaurants as a substantial amount of sodium can come from salt added during food processing. A cross-sectional study of dietary intake in the CKiD cohort revealed that fast foods were the largest single source of sodium, contributing 9.4% of the total intake; the dietary sodium intake increased with age and exceeded the recommended maximum intake in all age groups [83]; the median sodium intake was 83 mg (3.6 mmol) per kg per day [120].

Infants receiving PD are particularly predisposed to substantial sodium losses, even when anuric. High ultrafiltration requirements per kilogram of body weight result in removal of significant amounts of sodium chloride. These losses are not adequately replaced through the low sodium content of breast milk (160 mg/L or 7 mmol/L) or standard commercial infant formulas (160–185 mg/L or 7–8 mmol/L) [121]. Therefore, infants on PD are at risk of developing hyponatremia that can result in cerebral edema and blindness and must be maintained in neutral sodium balance; this is discussed further in ► Chap. 75, "Peritoneal Dialysis."

Potassium Potassium homeostasis in children with CKD is usually unaffected until the GFR falls to <10% of normal. However, children with renal dysplasia, post-obstructive kidney damage, reflux nephropathy, and renal insufficiency secondary to interstitial nephritis often demonstrate renal tubular resistance to aldosterone and may manifest hyperkalemia, even when their GFR is relatively well preserved. The hyperkalemia experienced by these children is exacerbated by volume contraction (and can be particularly common in salt losers) and the majority of the patients respond to salt and water repletion. In patients who are persistently hyperkalemic, dietary potassium intake should be limited. As potassium content is infrequently listed on food labels, a list of foods rich in potassium such as chocolates, French fries, potato chips, bananas, green leafy vegetables, dried fruits, and orange juice should be provided to patients and their families. Altering the methods of food preparation, such as soaking vegetables before cooking, helps decrease potassium content. Moderate to severe hyperkalemia may require treatment with a potassium binder such as sodium polystyrene sulfonate (SPS) (Kayexalate®), Patiromer (Veltassa®), or sodium zirconium cyclosilicate (SZC) (Lokelma®). In contrast to SPS that exchanges sodium for potassium ions and can also bind calcium and potentially result in hypocalcemia, Patiromer exchanges calcium for potassium ions, thus avoiding the provision of a sodium load and the possible inadvertent development of hypocalcemia. At present, Patiromer and SZC have only been approved for usage in adults and pediatric trials are ongoing.

In the case of infants and young children, special milk formulas such as Renastart® (Vitaflow USA) or Renalcal® (Nestle Health Sciences) are low in potassium and can be used to minimize exposure to dietary potassium (Table 6) [122].

Alternatively, the potassium content of formula can be reduced by pretreating it with a potassium binder, SPS, or Patiromer [123]. It is important to note that pretreatment of milk formula with SPS can reduce the calcium concentration by 8–29% and increase the sodium concentration by 86–300% [124].

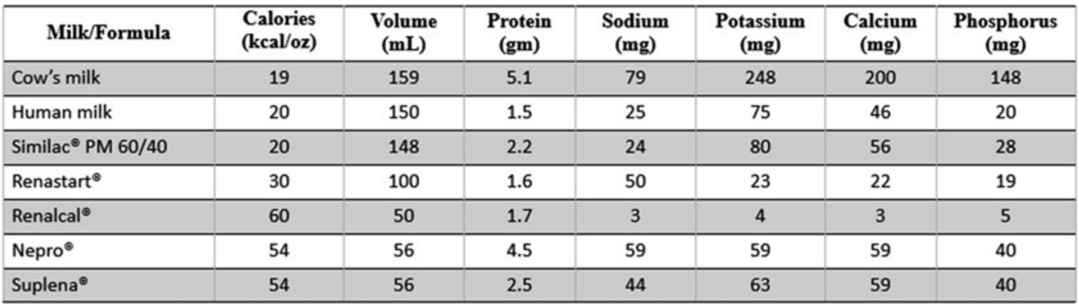
In children receiving maintenance HD, dietary potassium intake should be distributed throughout the day, as high serum concentrations of potassium can develop when a large quantity of potassium is ingested at one time, regardless of the total daily dietary content. On the other hand, some patients receiving PD may become hypokalemic due to potassium losses in dialysate and will require potassium supplementation, as hypokalemia has been associated with an increased risk for the development of peritonitis. Finally, constipation should be treated aggressively as significant quantities of potassium are eliminated through the gastrointestinal route in patients with CKD.

Vitamins and Micronutrients

Vitamins and minerals are essential for normal growth and development and either a deficiency or an excess can prove harmful. Unfortunately, the vitamin and mineral needs (other than for Vitamin D) of pediatric patients with CKD are not clearly defined, and the limited data that is available is derived from patients undergoing maintenance dialysis. Children with CKD are prone to develop vitamin deficiencies because of anorexia and dietary restrictions, while they are also at risk to develop toxic levels of vitamins when the renal clearance is significantly impaired.

All of the water-soluble vitamins except pyridoxine are eliminated by the kidneys and their clearance in patients with CKD is not known. However, most water-soluble vitamins are lost during maintenance dialysis and, in turn, should be routinely supplemented by special vitamin formulations that do not contain vitamin A and D, such as Nephronex® (L Lorens Pharmaceuticals), Nephro-Vite® (Allergan Inc.), and Dialyvite® (Hillestad Pharmaceuticals Inc.). Vitamin B_{12} and folic acid are both important for effective erythropoiesis, and daily supplementation with 0.8–1.0 mg folic acid is routinely recommended, while the necessity of vitamin B_{12} supplementation remains unsettled; this is discussed further in ▶ Chap. 73, “Anemia in Chronic Kidney Disease.” A higher dose of folic acid (2.5 mg per day) has been suggested by some for children with CKD and on dialysis, as supplemental folic

Table 6 Milk and selected commercial formula nutrient content per 100 kcal [122]



Milk/Formula	Calories (kcal/oz)	Volume (mL)	Protein (gm)	Sodium (mg)	Potassium (mg)	Calcium (mg)	Phosphorus (mg)
Cow's milk	19	159	5.1	79	248	200	148
Human milk	20	150	1.5	25	75	46	20
Similac® PM 60/40	20	148	2.2	24	80	56	28
Renastart®	30	100	1.6	50	23	22	19
Renalcal®	60	50	1.7	3	4	3	5
Nepro®	54	56	4.5	59	59	59	40
Suplena®	54	56	2.5	44	63	59	40

Conversion mg to mEq: sodium 23 mg = 1 mEq; potassium 39 mg = 1 mEq. Human milk electrolyte composition can vary with maternal diet. Renalcal® is a calorically dense, minimized electrolyte formula which should not be used as the only source of complete feeding acid has been shown to decrease the elevated homocysteine levels that are commonly seen in patients with CKD and that is a potential risk factor for cardiovascular morbidity and mortality [125].

Vitamin A levels are usually elevated in children with CKD as a result of the loss of the kidneys' normal ability to excrete vitamin A metabolites. Since elevated levels of vitamin A can be associated with the development of hypercalcemia and complications related to a high calcium-phosphorus product, it is important to avoid the use of vitamin supplements that include vitamin A.

Aluminum, copper, chromium, lead, strontium, tin, and silicon levels have all been noted to be elevated in patients with CKD, reflecting the fact that their clearance is dependent on an adequate GFR [126]. In contrast, zinc levels have been shown to be low in malnourished children and should be monitored and supplemented as necessary.

Current KDOQI pediatric nutrition guidelines [33] recommend the intake of at least 100% of the DRI for thiamin (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_8), cobalamin (B_{12}), ascorbic acid (C), retinol (A), α-tocopherol (E), vitamin K, folic acid, copper, and zinc for children with CKD stages 2–5, and those receiving maintenance dialysis. In children older than 6 years of age undergoing PD, vitamin supplementation has been associated with normal or greater than normal serum levels of the water-soluble vitamins [127]. As most infant milk formulas including Similac PM 60/40 are fortified with both water-soluble and fat-soluble vitamins, most infants with CKD/ESKD receive the DRI/RDA for all vitamins (including vitamin A) by dietary intake alone.

Carnitine Carnitine is essential for normal mitochondrial function, fatty acid and energy metabolism, and cell membrane stability. Carnitine is essentially found in two forms in the body, as free carnitine and as acylcarnitines (compounds in which carnitine is bound, by acyl-binding, to fatty acids of varying size). While the serum total (sum of free carnitine and acylcarnitines), free, and acylcarnitine concentrations in the CKD patients are not different from the values in healthy individuals [128], in patients receiving HD, the serum free carnitine concentration is significantly lower because of increased removal during dialysis, and the serum acylcarnitine concentration is significantly greater due to decreased clearance; as a result, the ratio of acylcarnitine to free carnitine, an index of abnormal carnitine metabolism, is dramatically increased in patients receiving HD [128].

Carnitine deficiency can result in the development of anemia, cardiomyopathy, and muscle weakness [129]. However, there currently is insufficient evidence to support the routine use of carnitine in either the pediatric CKD or dialysis patient populations. However, a trial of supplemental carnitine may be indicated when all other causes for the symptoms in question have been excluded, carnitine deficiency has been confirmed, and the patient has been unresponsive to standard therapies [130]. Carnitine deficiency is reflected by an acyl: free carnitine ratio greater than 0.4 (i.e., [total – free carnitine] ÷ free carnitine) or a total serum carnitine value less than 40 μmol/L [129].

Nutrition Management

A registered dietitian with experience in pediatric kidney diseases should play the central role in the dietary management of children with CKD/ESKD. In addition to possessing knowledge related to nutritional requirements, this person should also be skilled in the evaluation of physical growth and the educational and social needs of this special population.

An individualized nutrition plan taking into account a variety of factors should be developed for each patient by the dietician in consultation with the physician, patient (when appropriate), and family, with clearly defined short- and long-term objectives. As cultural food preferences play an important role in the family's ability to adhere to dietary changes, dietary instructions should be tailored to help families modify, but not eliminate cultural food preferences. Background information on cultural diets and translated versions of renal diets and food lists are available for reference [131]. The plan should be modified as necessary according to changes in the child's nutritional status, kidney function, dialytic therapy, medication regimen, and psychosocial situation, with particular attention paid to any concerns for food insecurity.

Dietary restrictions should be limited as much as possible with a goal of enhancing nutrient intake. It is also important to find substitutes for restricted foods so as to maintain an adequate caloric intake. Adopting and maintaining changes in eating habits is also easier for a child if family members make similar changes, or at least avoid eating restricted foods in the child's presence. While the ideal goal is full compliance with the prescribed regimen, it is not always a realistic expectation and "partial compliance" is often acceptable. Being very rigid with the dietary prescription adds to parental stress and increases the risk for behavioral eating problems in the young child such as food refusal, gagging, and vomiting.

Oral Supplementation

Infants with CKD requiring fluid restriction or those who have a poor oral intake may require a greater caloric density of their milk formula than the standard 20 kcal/oz. The increase in caloric density should not be achieved by concentrating the milk formula, as this approach will also increase the protein and mineral content. The provision of extra calories can be achieved by adding carbohydrate and/or fat modules to the formula. Glucose polymers such as Polycose® (Abbott Nutrition) or SolCarb® (Medica Nutrition) have a low osmolality and are generally the initial supplements added to infant formulas. Additional calories can be added in the form of corn oil. Microlipid® (Nestle Nutrition), a 50% fat emulsion from safflower oil with 4.5 kcal/mL, and Duocal® (Nutrica North America), a fat and carbohydrate combo modular with 5 kcal per gram of powder, are commercially available products for energy supplementation. It is important to wait at least 24 h following each 2- to 4-kcal/oz incremental increase in concentration to enhance patient tolerance of the formula.

A variety of calorie-dense (1.8 kcal/mL) preparations such as Nepro® and Suplena® (Abbott Nutrition) have been formulated specifically for patients with kidney disease. Suplena® has a lower protein content than Nepro® (30 g/L vs. 70 g/L) and is preferable for pre-dialysis patients. These preparations are characterized by a low renal osmolar load and a low vitamin A and D content. Although initially produced for patients older than 10 years, they have been used in children as young as 3 years; however, it is advisable to dilute them to half to two-third strength when used in young children.

In contrast to energy intake, the protein requirements of children with CKD are usually met by voluntary, unsupplemented consumption. A recently published CKiD experience revealed that the median dietary protein intake of children with CKD exceeded recommended levels in all age groups, particularly among younger subjects [83]. However, if on occasion the protein intake is insufficient due to concomitant phosphorus restriction in a patient with severely impaired kidney function, the protein module, Beneprotein® (Nestle Nutrition), a whey protein concentrate, can be added to the formula to increase protein content. As much as 1 gm of Beneprotein®, which is equivalent to 0.86 gm protein, can be added to each ounce of formula.

Enteral Nutritional Support

Aggressive enteral feeding should be considered if nutritional intake by the oral route is suboptimal. The use of enteral support has resulted in maintenance or improvement of SD scores for weight and/or height in infants and young children with moderate to severe CKD and those undergoing maintenance dialysis [85, 86, 132].

Nasogastric (NG) tubes, gastrostomy tubes, gastrostomy buttons, and gastrojejunostomy tubes have all been used to provide supplemental enteral feeding to children with kidney disease [133]. A study from the IPPN registry, however, revealed that tube feeding was predominantly used in North America and Europe, with very little usage elsewhere in the world [85] [86]. The tube feeding can be given as an intermittent bolus, or more commonly by continuous infusion during the night. Continuous overnight feeds are generally preferred to allow time during the day for regular oral intake. Historically, the NG tube was used most frequently in infants and young children, as it is easily inserted and is generally well tolerated [132]. However, this route of therapy is often complicated by recurrent emesis and the need for frequent tube replacement, in addition to the risk of pulmonary aspiration, nasoseptal erosion, and psychological distress of the caretaker because of the cosmetic appearance. An NG tube is, however, the preferred option for short-term enteral feeding, and may be considered as a bridging option to a long-term enteral feeding tube. Persistent emesis can be addressed by slowing the rate of formula delivery and by the addition of antiemetic agents such as metoclopramide or domperidol. Additionally, whey-predominant formulas can be used as they have been shown to stimulate gastric emptying [134].

The gastrostomy tube or button is now considered the standard approach to long-term enteral feeding. It has the cosmetic advantage of being hidden beneath clothing. The reported complications of gastrostomy tubes/buttons include exit-site infection, leakage, obstruction, gastrocutaneous fistula, and peritonitis in patients receiving PD [135]. To decrease the risk of peritonitis, the gastrostomy should be placed either before or simultaneously during PD catheter placement, accompanied by antibiotic and antifungal prophylaxis [33, 136]. In addition, it may be better to avoid combining gastrostomy placement and peritoneal dialysis catheter placement in a severely malnourished patient until the nutritional status and general immunity of the patient can be improved by other means, such as NG tube feeds.

Transpyloric tubes are recommended only in circumstances of severe gastroesophageal reflux, gastroparesis, or gastric outflow obstruction. Positioning of the tube within the jejunum requires radiological guidance. As boluses entering the jejunum are poorly tolerated, both gastro-jejunal and naso-jejunal tubes require that all formula delivery is by continuous infusion.

A common and serious complication of using any form of enteral tube feeding is a prolonged and potentially difficult transition from tube to oral feeding. Regular non-nutritive sucking and repetitive oral stimulation are recommended for all tube-fed infants.

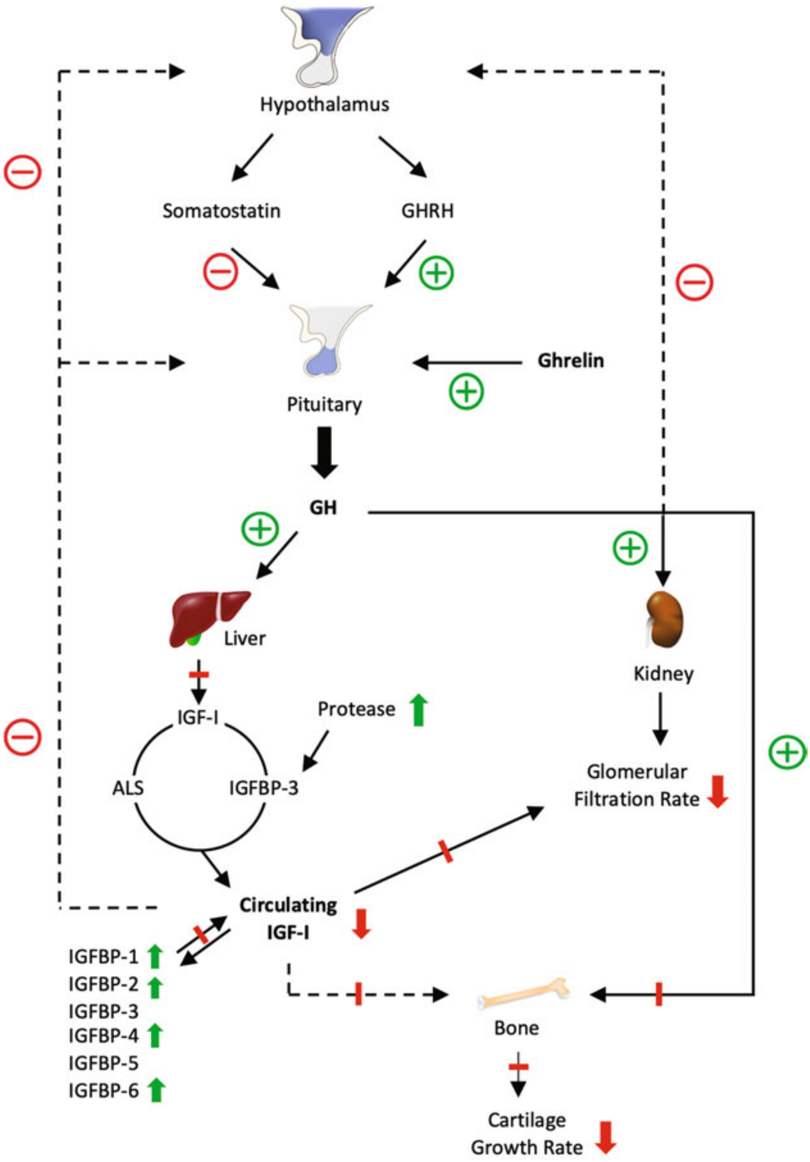
Growth Hormone-Insulin Growth Factor (IGF)-I in CKD

Growth hormone (GH) is a 22-kD protein which is filtered by the glomerulus and plasma levels increase as kidney function worsens due to increased secretion and reduced clearance of GH. GH acts via the GH receptor to stimulate the synthesis of insulin growth factor-1 (IGF-I) at growth plate cartilage and in the liver from where it is liberated into the circulation. Despite normal IGF-I levels in children with CKD, IGF-I synthesis and bioactivity are decreased in CKD and this fact provides the rationale for recombinant human growth hormone (rhGH) therapy. The impact of CKD on growth hormone receptor density, growth hormone function, and subsequent growth is not clear. Some studies demonstrate a lower density of GH receptors in the liver and reduced hepatic GH receptors have been demonstrated in animal studies [137]. Another proposed mechanism for GH and IGF-I resistance is a post-receptor GH signaling defect which has been repeatedly observed in key tissues of CKD rats [16]. Signal transduction following GH binding to its receptor requires the activation of JAK2, which in turn stimulates tyrosine kinase to phosphorylate STAT1, STAT3, and STAT5. As soon as these are phosphorylated, they form dimers which pass into the nucleus, activating genes that generate IGF-I. Impaired JAK2–STAT5 phosphorylation has been demonstrated to play a part in GH resistance and growth failure in CKD rats [16]. Another family of proteins, SOCS, contributes to the GH signaling as upregulated SOCS dephosphorylate STAT, leading to a negative feedback action of GH in CKD [16]. CKD patients also exhibit reduced levels of circulating growth hormone–binding protein (GHBP). Higher GHBP levels are associated with improved growth patterns and with a positive response to rhGH treatment in pediatric CKD [137].

Other mechanisms for GH resistance in CKD include effects on IGF-I activity. Serum total IGF-I levels in children with mild to moderate CKD are adequate, but are decreased in those with ESKD. In addition, as CKD progresses the level of free IGF-I is diminished and IGF-I activity decreases [138]. This discrepancy is attributed to increased levels of IGF-binding proteins due to decreased kidney clearance in CKD leading to a decrease in the active form of IGF-I (Fig. 3) [138–141].

Chronic metabolic acidosis negatively impacts growth in children with CKD as well, through alterations in the GH–IGF-I axis and response to IGF-I [141]. Several studies have shown that acidotic rats grew less and had a significantly smaller size of the tibial epiphyseal growth plates compared with matched controls. These data indicate that metabolic acidosis induces resistance to IGF-I

Fig. 3 GH-IGF-I in CKD. Molecular and endocrine perturbations in GH-IGF axis contributing to GF in CKD [139]


Hypothalamus
Somatostatin
GHRH
Pituitary
Ghrelin
GH
Liver
Kidney
IGF-I
Protease
ALS
IGFBP-3
Glomerular Filtration Rate
Circulating IGF-I
IGFBP-1
IGFBP-2
IGFBP-3
IGFBP-4
IGFBP-5
IGFBP-6
Bone
Cartilage Growth Rate

action within the tibial epiphyseal growth plate [142].

Growth Hormone Therapy in Pediatric CKD

In pediatric CKD patients, the resistance to GH can be overcome by supraphysiologic doses of rhGH that significantly enhance growth velocity and potentially result in a more normal adult height [143]. Numerous studies have shown the safety and efficacy of rhGH in pediatric CKD, even after kidney transplantation [144]. A Cochrane review which included 16 controlled trials of rhGH therapy in 809 pediatric patients in various stages of CKD showed that children who received 1 year of rhGH had a higher average HtSDS and height velocity as compared with controls, and that rhGH therapy significantly improved HtSDS and height velocity in this population [145].

A recent consensus statement from the European Society for Pediatric Nephrology CKD Working Group recommended rhGH treatment in pediatric CKD for patients with a HtSDS <3rd percentile or with a height velocity < -2 SD. The consensus statement warns that rhGH therapy should be started only after other contributing factors to growth failure have been appropriately managed, including treatment of anemia and infections, correction of nutritional deficiencies, and optimization of CKD-MBD management [146]. The KDOQI nutritional guidelines recommend that rhGH should be considered if catch-up growth has not been triggered after 3 months of appropriate nutritional management [33].

Administration of 28–30 IU rhGH/m^2 per week or 0.05 mg/kg/day seems to be the optimal dose in most studies. A doubling of this dose did not result in significant improvement in the growth of transplanted patients. Several studies have indicated a more robust response to rhGH in prepubertal children than during adolescence. The improvement of growth was negatively correlated with patient height at treatment initiation, and the most stunted patients responded best to rhGH treatment. Another important factor which influences the growth response is the primary renal disorder. Children with renal hypo/dysplasia, which is by far the most frequent primary renal disease leading to CKD in children, responded better than children with glomerulonephritis or other renal disorders [147]. Finally, a study including 836 adult or near-adult patients on various modalities of maintenance dialysis who received rhGH during childhood for at least 2 years showed a median absolute increase in height of 7.4 cm in boys and 7.0 cm in girls [143].

Several randomized controlled trials and observational studies have shown that while rhGH is safe and well tolerated, long-term follow-up data are still lacking. Potential adverse effects include increased intracranial hypertension, increased incidence of malignancy, slipped capital femoral epiphyses (SCFE), avascular necrosis, glucose intolerance, pancreatitis, allograft rejection, and fluid retention. Whereas a Cochrane review showed that the prevalence of adverse events related to use of rhGH was usually similar in the intervention and control groups, screening and monitoring for these side effects remains important (Table 7) [145, 146].

Table 7 Recommended monitoring in CKD patients receiving rhGH therapy

Baseline fundoscopic exam due to concern for risk of increased intracranial pressure
Glucose intolerance, especially in patients with nephropathic cystinosis who are at higher risk for diabetes
Secondary hyperparathyroidism which may be accelerated or discovered during GH treatment. Withhold rhGH if iPTH is >500 pg/mL
SCFE, which is more likely to occur during periods of rapid growth and if iPTH >500 pg/mL. X rays should be obtained if symptoms develop
Renal disease progression
Bone and sexual maturation with Tanner staging and annual wrist radiographs
Growth response; discontinue use if inadequate response after 6 months, renal transplantation, patient attains genetic height or epiphyseal growth plate closure is evident on radiographs

Although its safety and effectiveness are well established, rhGH remains an underutilized treatment option. There is considerable variation in pediatric nephrology practices in addressing growth failure with rhGH use in children with CKD, and patient nonadherence to rhGH therapy as a result of the need for daily injections is frequent [148]. In the CKiD study, the rhGH nonadherence rate was about 18% and, as expected, nonadherence to rhGH was associated with poorer growth velocity, indicating a possible window of opportunity for an intervention to improve CKD patient outcome [149].

Neurocognitive Development

Brain development is most rapid during early childhood, and thus the developing brain is at greatest risk for injury during infancy and early childhood [150]. In turn, several studies have suggested that there is an increased risk for developmental delay in children with early onset CKD or ESKD [151, 152].

There are a number of factors associated with impaired kidney function that may contribute to impaired neurocognition. In addition to the potential direct adverse effects of metabolic neuronal toxicity, vascular injury and endothelial dysfunction, malnutrition, aluminum toxicity, and psychosocial deprivation have all been associated with neurocognitive dysfunction in children with CKD and ESKD [153]. Thankfully, recognition of the adverse impact of the latter three factors have led to alterations in patient management so as to ideally minimize or even eliminate their influence on neurocognition [49, 154].

Early studies of neurodevelopment in children with CKD/ESKD, at a time when the connection between malnutrition, aluminum intoxication, and potentially dialysis adequacy with neurocognition was less recognized, provided evidence of severe delays in subjects both on and not yet on dialysis [155]. While the published studies were characterized by small sample sizes, and patients of a wide age range and receiving a variety of different treatment regimens, delays in gross motor function and language and lower IQ scores were well documented, as was the presence of microcephaly in the youngest patients [156]. Older children and adolescents have also demonstrated significantly lower IQ scores compared to controls, with evidence of poorer abilities in gross and fine motor coordination, visuomotor skills, verbal abilities, attention, and short-term memory, the latter deficit being greatest in those children on dialysis [155, 157]. Some, but not all, studies have also revealed abnormalities of executive function, as well as problems in academic achievement as reflected by difficulties with reading, spelling, and math, all of which are associated with increased school absences and more frequent grade retention [158]. In addition, these issues may translate into lower verbal and nonverbal capacities and executive dysfunction in adulthood. Electrical conduction dysfunction with EEG abnormalities have also been reported in children with CKD from infancy, abnormalities which may contribute to the development of a seizure disorder, a complication noted in 0–20% of children with ESKD. While the receipt of a kidney transplant has repeatedly been shown to be associated with improvements in cognitive function, most beneficial to long-term neurocognitive outcome appears to be a shorter duration of dialysis during childhood and older age at the time of renal replacement therapy [159, 160].

Most important to the management of children with CKD/ESKD from the neurocognitive perspective is regular surveillance by the medical team, in concert with the child's family, with recognition of the increased risk that exists for neurodevelopmental challenges. If detected, the institution of developmentally appropriate interventions designed to optimize the child's cognitive trajectory should be carried out, in addition to the necessary medical interventions, and coordinated by a multidisciplinary team which includes representatives from nephrology and psychology at a minimum, with other resources (e.g., neurology, physical/occupational therapy, etc.) as deemed appropriate.

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Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD)

72

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Abstract

Chronic kidney disease mineral and bone disorder (CKD-MBD) is a systemic disorder that encompasses alterations in mineral homeostasis, bone turnover, mineralization, volume and strength, linear growth and strength, and cardiovascular disease. CKD-MBD in childhood may lead to significant bone pain, deformities, and fractures that hinder the daily activities and quality of life of children with advanced CKD; these sequelae persist into adulthood whereas many as 18% of this population reported disabilities resulting from their bone disease. Children with CKD, especially those on dialysis, have a greatly increased risk of cardiovascular (CV) morbidity and mortality; vascular calcifications are present in even children and young adults with end-stage kidney disease (ESKD). Management of CKD-MBD requires attention to long-term skeletal and cardiovascular health. Clinical evaluation of height, growth velocity, blood pressure and a musculoskeletal examination, focusing on bone pain, deformities, and fractures, should be performed routinely in children with CKD; dietary calcium (Ca) and phosphate (P) intake should also be assessed at regular intervals. P-binders and vitamin D analogs should be used to treat secondary hyperparathyroidism in children with advanced CKD. Age-, gender-, and CKD stage- appropriate values of Ca, P, alkaline phosphatase, and PTH should be used to guide therapy.

Keywords

Chronic kidney disease mineral and bone disorder (CKD-MBD) · Renal bone disease · Calcium (Ca) · Phosphate (P) · Parathyroid hormone (PTH) · Vitamin D · Phosphate binders · Vascular calcification · Secondary hyperparathyroidism

Introduction: CKD-MBD in Pediatrics

Chronic kidney disease mineral and bone disorder (CKD-MBD) is a systemic disorder that encompasses alterations in mineral ion homeostasis, in bone turnover, mineralization, volume and strength, linear growth and strength, and cardiovascular disease [1]. This chapter describes the epidemiology, pathophysiology, and treatment of these alterations in children with all stages of CKD and a variety of CKD etiologies.

Epidemiology

Skeletal symptoms, including limb deformities, bone pains and fractures, or radiological signs of bone disease have been reported in 15% of children on peritoneal dialysis [2]. This number likely underestimates the true prevalence of clinical bone disease in childhood CKD, and a recent study reported that significant bone pain hindered the daily activities of 58% of children with advanced (i.e., stages 4–5) CKD [3]. In large prospective studies of children with CKD, including the North-American Chronic Kidney Disease in Childhood (CKiD) cohort, a 2.4- and 3-fold higher rate of fractures have been reported in boys and girls (respectively) with pre-dialysis CKD as compared to healthy children [4]. Fracture risk in pediatric CKD is associated with baseline-walking difficulty, more advanced (Tanner stages 4–5) pubertal development, lower height Z-score, higher parathyroid hormone (PTH) levels, lower Ca and 25-hydroxyvitamin D (25D) levels and team sports participation [4]. Phosphate binder (P-binder) use (and particularly Ca-based P-binders) have been associated with decreased fracture risk in children with pre-dialysis CKD, suggesting that improved P control and/or increased calcium intake may exert some protective benefit [70]. Importantly, CKD-MBD in childhood leads to important sequelae in adulthood, with 37% of Dutch adults who developed end-stage kidney disease (ESKD) before 14 years of age reporting severe bone disease (defined by bone deformities, chronic pain, low-trauma fracture, and/or aseptic necrosis) and 61% reporting severely decreased final adult height. As many as 18% of this population reported disabilities resulting from their bone disease [4].

In addition to skeletal disease, children with CKD, especially those on dialysis, have a 10-fold increase in cardiovascular (CV) morbidity and mortality; vascular calcifications are present in even children and young adults with ESKD [5–7].

Pathophysiology of CKD-MBD

A complex interplay between kidney, gut, bone, and parathyroid glands drives the pathogenesis of CKD-MBD. As glomerular filtration rate (GFR) declines, a cascade of maladaptive events ensue that result in bone disease, extra-skeletal calcification, and adverse cardiovascular outcomes as well as impaired quality of life and decreased life expectancy. Disordered regulation of Ca and P, increasing skeletal resistance to the actions of PTH, an impaired growth hormone – insulin-like growth factor 1 (GH/IGF1) axis, malnutrition (either under- or over-nutrition), iron deficiency, acidosis, uremic toxins, altered signaling in pathways driving bone and vascular biology (e.g., receptor activator of nuclear factor kappa-B [RANK] and RANK-ligand [RANKL], bone morphogenic protein [BMP]; runt-related transcription factors [RUNX] and wingless and integrin 1 [WNT], etc.), and underlying genetic mutations directly affect both bone and vessels. In addition, the therapies used to treat CKD-MBD affect not only mineral ion homeostasis but osteoblast/osteoclast/osteocyte cross-talk in bone and vascular smooth muscle cell biology (Fig. 1).

Bone consists primarily of Ca and P, in the form of hydroxyapatite; thus, the alterations in mineral metabolism that occur with progressive CKD lead to bone disease. While bone is the major reservoir for P and Ca ions, hormones derived from bone cells, the parathyroid glands, and renal proximal tubular cells (i.e., fibroblast growth factor 23 [FGF23]; parathyroid hormone [PTH]; and 1,25-dihydroxyvitaminD [1,25D], respectively) all regulate Ca and P homeostasis. Circulating FGF23 levels increase in very early CKD, due to decreased renal FGF23 clearance, an early decline in renal Klotho (a critical FGF23 co-factor in most tissues), and CKD-mediated changes in bone biology which stimulate skeletal FGF23 expression [8]. Regardless of its primary cause, increased circulating FGF23 levels suppress renal 1α-hydroxylase activity, causing 1,25D levels to fall in even early CKD stages. Decreased 1,25D levels in turn reduce intestinal Ca absorption. The combination of low 1,25D and low ionized Ca levels stimulate PTH release from the parathyroids, resulting in secondary hyperparathyroidism. Approximately 50–60% of patients with late CKD stages (CKD stages 4 and 5) develop hypocalcemia in response to the decreased intestinal Ca absorption that develops from critically low calcitriol concentrations; decreased P excretion (from critically low renal mass) contributes to hyperphosphatemia in these patients with severe CKD [9]. In addition, 25D deficiency, which is prevalent worldwide, also contributes to the development of secondary

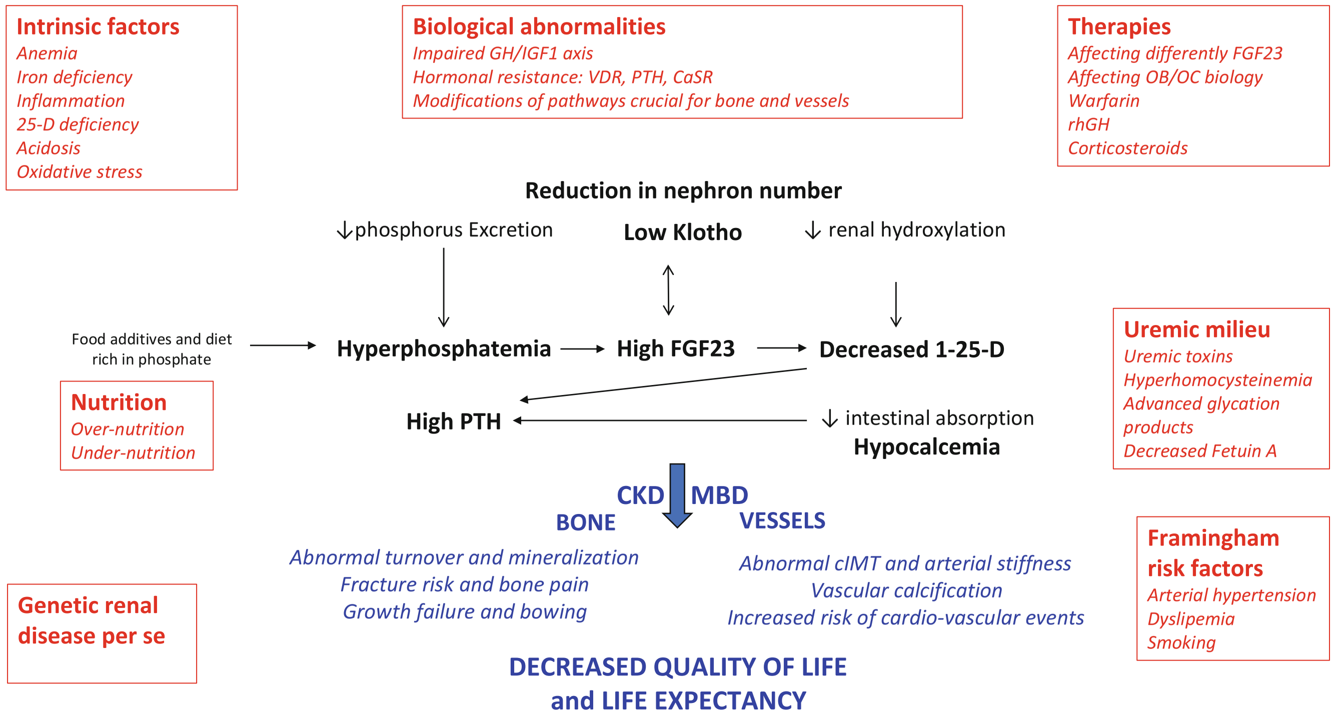
Intrinsic factors
Anemia
Iron deficiency
Inflammation
25-D deficiency
Acidosis
Oxidative stress
Biological abnormalities
Impaired GH/IGF1 axis
Hormonal resistance: VDR, PTH, CaSR
Modifications of pathways crucial for bone and vessels
Therapies
Affecting differently FGF23
Affecting OB/OC biology
Warfarin
rhGH
Corticosteroids
Reduction in nephron number
↓phosphorus Excretion
Low Klotho
↓ renal hydroxylation
Food additives and diet rich in phosphate
Hyperphosphatemia
High FGF23
Decreased 1-25-D
Nutrition
Over-nutrition
Under-nutrition
High PTH
↓ intestinal absorption
Hypocalcemia
Uremic milieu
Uremic toxins
Hyperhomocysteinemia
Advanced glycation products
Decreased Fetuin A
CKD MBD
BONE
VESSELS
Abnormal turnover and mineralization
Fracture risk and bone pain
Growth failure and bowing
Abnormal cIMT and arterial stiffness
Vascular calcification
Increased risk of cardio-vascular events
Framingham risk factors
Arterial hypertension
Dyslipemia
Smoking
Genetic renal disease per se
DECREASED QUALITY OF LIFE and LIFE EXPECTANCY

Fig. 1 **Overview of pediatric CKD-MBD**. The reduction in nephron number decreases both P reabsorption and renal 1-alpha hydroxylation of vitamin D, thus inducing hyperphosphatemia, decreased 1–25 $(OH)_2$ levels and subsequent hypocalcemia. All these factors increase PTH levels, with the development of secondary hyperparathyroidism. Dysregulated mineral metabolism is central to the vasculopathy and bone disease of CKD but additional factors worsen this (already complex) CKD-MBD setting, as illustrated around the elementary and fundamental factors explaining the pathophysiology of CKD-MBD. *OB/OC/OCy* osteoblast/osteoclast/osteocyte, *rhGH* recombinant human growth hormone, *cIMT* carotid intima/media thickness

hyperparathyroidism; a placebo-controlled randomized trial in children with pre-dialysis CKD demonstrated that ergocalciferol therapy is able to delay the onset of secondary hyperparathyroidism in this population [10]. Although data from the CKiD population suggest that increased P load is not an important driver for early increases in FGF23, a decline in circulating P concentrations can be observed in very early CKD when circulating FGF23 levels and bone FGF23 expression are increased [11]; hyperphosphatemia does contribute to increased FGF23 in late CKD. In later stages of CKD, treatment with active vitamin D analogs further contribute to increasing FGF23 levels. Non-mineral metabolism factors such as iron status, erythropoietin, and inflammation may also contribute to increased FGF23 production in all CKD stages. In murine models, both absolute and “functional” iron deficiency increase bone FGF23 expression [12]. Erythropoietin can also stimulate FGF23 production and, conversely, FGF23 may suppress erythropoiesis [13]. FGF23 levels are higher in patients with glomerular diseases when compared to those with congenital anomalies of the kidneys and urinary tract (CAKUT) [14]; inflammation increases bone and circulating FGF23 levels [12].

Normal Bone Development

Bone Structure

Bone provides structure and support for the body, protecting organs and providing levers for movement. Bone also acts as a reservoir for the storage and release of chemicals such as Ca and P that are critical for cellular function. In addition, bone acts as an endocrine organ, making and secreting factors that act locally, on its own cells [15] as well as on distant organs that regulate mineral ion homeostasis [16] and energy metabolism [17].

Bone tissue is woven into two main patterns: cortical and cancellous bone. Cortical bone is the major component of the long bones in the appendicular skeleton but also surrounds the exterior portion of bones in the axial skeleton. Cancellous bone is the internal tissue of skeletal bone which is found at the ends of long bones, near joints, and in the interior of vertebrae and comprises 20% of total bone mass in adults. Cancellous bone is more metabolically active than is cortical bone, accepting mineral ions from, and releasing them to, the circulation in response to hormonal stimuli [18].

As early as weeks 3–4 of embryonic development, somites form from the paraxial mesoderm, establishing the axial body structure. Most of the skeleton (including the vertebrae and the limb long bones) is formed by endochondral ossification in which an initial cartilage template is replaced by calcified bone (Fig. 2) [19]. Osteoblasts derived from chondrocytes and from mesenchymal cell within the bone collar replace cartilage matrix with a matrix rich in type I collagen. This collagen serves as a nidus for Ca and P – in the form of hydroxyapatite – accumulation. Intramembranous bone formation is critical for the patterning and ossification of the skull and clavicle. In this form of bone development, neural crest-derived mesenchymal cells proliferate and differentiate into osteoblasts, which secrete collagen-proteoglycan matrix that can bind Ca salts and vascular cells [20]. Skeletal patterning is complete and the basic shapes of all bones are established by 9 weeks postconception [21]. After this time, the skeletal matrix accumulates mineral (ossifies) and grows, but does not change its basic external shape.

Growth of long bones involves lengthening and also an increase in diameter. Lengthening involves growth followed by osteoblastic transition and ossification of chondrocytes in epiphyseal cartilage [22] while bone widening requires the apposition of tissue to the external surface by osteogenic cells in the periosteum. During bone modeling, the internal structure of bone is modified; trabecular thickness increases by about 30% during the second half of gestation while cortical thickness at the mid-shaft doubles [23]. After birth, periosteal bone formation and endosteal bone lengthening both continue. In adulthood, cyclical episodes of periosteal bone formation are coupled to episodes of bone resorption in the process of bone remodeling; however, periosteal bone apposition exceeds bone resorption in children (i.e., bone modeling) [24].

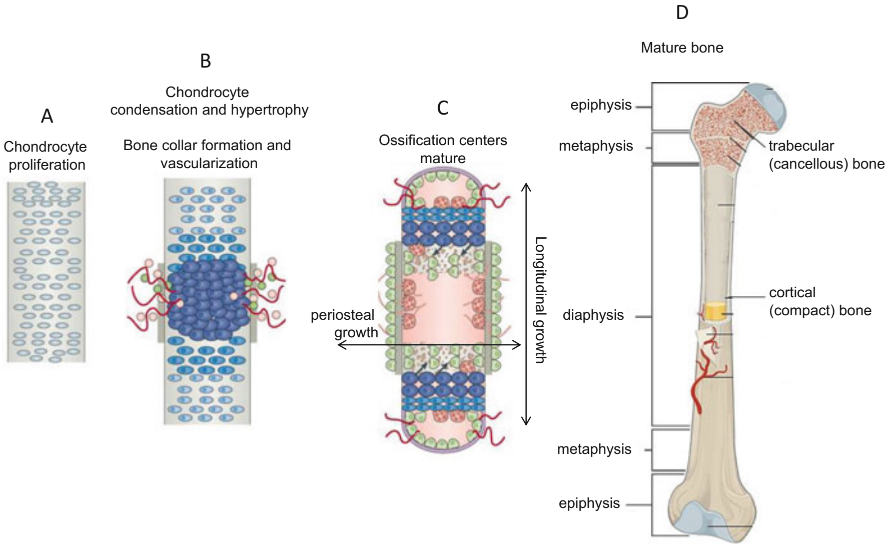
A
Chondrocyte proliferation
B
Chondrocyte condensation and hypertrophy
Bone collar formation and vascularization
C
Ossification centers mature
periosteal growth
Longitudinal growth
D
Mature bone
epiphysis
metaphysis
diaphysis
metaphysis
epiphysis
trabecular (cancellous) bone
cortical (compact) bone

Fig. 2 **Endochondral bone formation:** (1) a matrix template.is created by chondrocytes which (**a**) proliferate. With time, chondrocytes condense and hypertrophy (**b**); A bone collar is formed and invaded by nutrient vessels. Bone growth (**c**) occurs by continued proliferation/hypertrophy/ossification at the ends of the bones (longitudinal growth) and by periosteal bone formation (periosteal/lateral growth). (**d**) Mature long bone is composed of a compact outer structure (cortical bone) and a cancellous (trabecular) inner structures of rods and plates

Role of Different Cell Types in Bone Structure and Function

Active remodeling of bone occurs throughout life through cross-talk between osteoblasts, which lay down bone matrix, and osteoclasts, which erode mature, mineralized bone. The function of both cell types is regulated by osteocytes embedded within mineralized bone. These coordinating signals create a tight feedback loop of bone formation and resorption in vivo*,* termed “coupling” [25].

Paracrine and Endocrine Functions of Bone Cells

Osteoblasts, osteocytes, and osteoclasts secrete proteins that regulate bone formation and remodeling in a local (paracrine) manner. Osteoblasts and osteocytes also have classic endocrine functions, secreting hormones which act on distal organs. Mature osteoblasts secrete large amounts of the non-collagenous protein osteocalcin (OCN; BGLAP), which is a major determinant of bone formation [26], and which also regulates metabolism in many tissues, increasing energy expenditure and reducing fat mass, improving insulin sensitivity, and preventing liver steatosis [27]. Osteocytes also have an important endocrine function, secreting FGF23 [28]. Although the factors that regulate osteocyte function are poorly defined, circulating mineral ions, such as P, PTH, and 1,25(OH)$_2$vitamin D, stimulate FGF23 expression. The stage of osteocyte maturation also appears to be critical in their function; FGF23 appears to be expressed almost exclusively in early osteocytes [8], while the Wnt-signaling inhibitor sclerostin is confined to the most mature osteocytes in bone [29].

Renal Bone Disease

Renal bone disease is a common complication of CKD and is nearly universal at the start of dialysis therapy. While the term “CKD Mineral and Bone Disorder” (“CKD-MBD”), refers to the alterations in mineral metabolism and their effects on both the cardiovascular and skeletal systems in the CKD population, the term “Renal osteodystrophy” is used specifically to describe the bone pathology that occurs as a complication of CKD [30]. On bone histology, renal osteodystrophy lesions are defined according to alterations in bone turnover, ranging from high bone turnover (secondary hyperparathyroidism, osteitis fibrosa) to lesions of low bone turnover (adynamic bone disease and osteomalacia). However, alterations in skeletal mineralization and volume are also common in pediatric CKD patients [31], and may contribute to outcomes such as fractures, skeletal deformities, and poor growth which persist despite normalization of bone turnover [4]. Each of these parameters is affected by circulating PTH, Ca, P, 25(OH) vitamin D, and FGF23 levels and also by renal osteodystrophy therapies [30]. However, as previously mentioned, uremia itself alters bone cell biology contributing to features such as PTH resistance and intrinsic mineralization defects [8]. These features limit the reliability of currently available biomarkers to predict underlying bone histology (Fig. 3).

The Bone Biopsy Procedure

Although iliac crest bone biopsy is an invasive procedure which is not universally performed, bone histomorphometry remains the gold standard for the diagnosis of different types of bone diseases associated with CKD [32]. Bone histomorphometry provides information about the histologic appearance and dynamics of bone formation and mineralization and can also inform the clinician on the accumulation of substances such as aluminum, oxalate, iron, and amyloid in bone. While not routinely recommended, a bone biopsy may be considered in all patients with CKD who have fractures with minimal trauma (pathological fractures), suspected aluminum

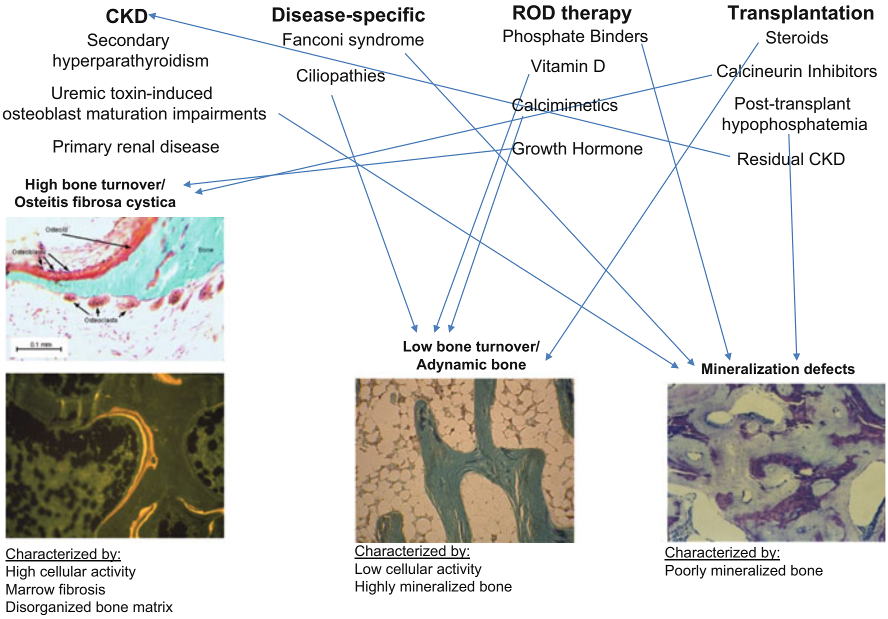
CKD
Secondary hyperparathyroidism
Uremic toxin-induced osteoblast maturation impairments
Primary renal disease
Disease-specific
Fanconi syndrome
Ciliopathies
ROD therapy
Phosphate Binders
Vitamin D
Calcimimetics
Growth Hormone
Transplantation
Steroids
Calcineurin Inhibitors
Post-transplant hypophosphatemia
Residual CKD
High bone turnover/ Osteitis fibrosa cystica
Osteoid
Osteoblasts
Bone
Osteoclasts
0.1 mm
Low bone turnover/ Adynamic bone
Mineralization defects
Characterized by:
High cellular activity
Marrow fibrosis
Disorganized bone matrix
Characterized by:
Low cellular activity
Highly mineralized bone
Characterized by:
Poorly mineralized bone

Fig. 3 Renal bone disease (renal osteodystrophy) comprises a spectrum of turnover, mineralization, and volume lesions. Untreated secondary hyperparathyroidism and medications such as calcineurin inhibitors promote high bone turnover, characterized by increased cellular activity and poorly organized bone matrix. Oversuppression of PTH with vitamin D sterols and calcium-based phosphate binders can lead to low bone turnover, which is characterized by highly mineralized bone with low cellular activity. Mineralization defects are common in CKD patients and are exacerbated by the direct effects of uremic toxins on osteoblast maturation and the overuse of phosphate binders

bone disease, or persistent hypercalcemia despite well-controlled PTH levels. Before the procedure, bone is labeled with tetracycline in order to evaluate bone turnover. A 2-day course of tetracycline is administered at 15 mg/kg/day (divided in twice or thrice daily doses). Fourteen days later, the 2-day course is repeated. For children younger than 8 years, tetracycline dosage is usually kept below 10 mg/kg/day to avoid toxicity.

Turnover

Abnormalities in bone turnover, driven by circulating PTH concentrations, are prevalent in children with advanced stages of CKD. PTH activates the PTH/PTHrP receptor on osteocytes and osteoblasts, thereby directly increasing the cellular activity of osteoblasts and, indirectly, the activity of osteoclasts [33]. Skeletal resistance to PTH develops as CKD progresses, due to multiple factors including, although likely not limited to the accumulation of circulating PTH fragments [34] and downregulation of the PTH receptor [35], complicating the interpretation of serum PTH levels in the assessment of bone formation rates. Prolonged exposure to elevated PTH levels and increases in bone turnover may lead to peritrabecular fibrous changes in bones; thus, the renal osteodystrophy associated with prolonged secondary hyperparathyroidism is often termed "osteitis fibrosa cystica." A state of low-turnover bone disease (adynamic renal osteodystrophy) defined as decreased bone formation rate in conjunction with decreased cellular activity and an absence of excessive osteoid accumulation may occur in children treated with maintenance dialysis who receive overaggressive therapy with active vitamin D sterols and Ca salts. In addition to the increased risk for fractures and vascular calcifications that is observed in adults with adynamic bone, this form of bone disease in children treated with dialysis is associated with a further decline in growth [36].

Although bone turnover is undoubtedly affected by changes in circulating PTH in advanced CKD, recent data from animals and humans have suggested that changes in bone turnover occur before alterations in circulating mineral, PTH, or vitamin D concentrations are apparent [31]. These changes are mediated, at least in part, by impaired osteoblast and osteocyte maturation [8] and by the increased expression of the osteocyte-specific Wnt-signaling inhibitor sclerostin [37] in all CKD stages.

Mineralization

Alterations in skeletal mineralization are also prevalent in children with CKD [31] and may be seen in children with early CKD, prior to the onset of secondary hyperparathyroidism. Defective mineralization appears to be the first detectably histologic lesion in children with CKD, affecting up to 40% of children with CKD stage 2 and as many as 80% of those with more advanced stages of CKD [31]. These lesions persist in children treated with maintenance dialysis and are not corrected by therapy with active vitamin D sterols [38].

Defective skeletal mineralization is associated with increases in unmineralized bone (osteoid) in conjunction with delayed rates of mineral deposition. In the face of low to normal bone turnover, defective mineralization in growing children is termed "rickets." The histomorphometric characteristics of rickets include the presence of wide osteoid seams, an increased number of osteoid lamellae, an increase in the trabecular surface covered with osteoid, and a diminished rate of mineralization or bone formation, as assessed by double tetracycline labeling. Fibrosis is absent. Defective mineralization in combination with increased bone formation rates is termed "mixed uremic osteodystrophy" and is characterized by wide osteoid seams, prolonged mineralization times, bone marrow fibrosis, and increased bone formation rates [30].

Although the mechanisms of skeletal mineralization are incompletely understood, factors such as 25(OH)vitamin D (25D) deficiency and altered FGF23 metabolism have been implicated in their pathogenesis. In the general population, nutritional 25D deficiency results in osteomalacia and a similar phenotype may occur in patients with

CKD. P depletion, as may occur with frequent dialysis and/or intensive use of P-binders, may also result in osteomalacia. FGF23 may also play a role; both overexpression [39] and ablation of FGF23 [28] in mice with normal renal function are associated with abnormal mineralization of osteoid, although by different mechanisms. The phosphaturic effect of increased FGF23 may cause rickets and osteomalacia through an insufficiency of mineral substrate. Osteomalacia in animals with FGF23 deficiency suggests that FGF23 may play a direct role in skeletal mineral deposition. Recent data suggest that factors that impair bone cell maturation also contribute to skeletal mineralization defects in pediatric CKD patients [8]. Elevated serum PTH levels are associated with mineralization defects in children with CKD, independent of abnormalities in bone turnover [31] and PTH plays an important role in osteocyte maturation [40]. While the ramifications of defective mineralization remain to be established, increased fracture rates and bone deformities are prevalent in patients with CKD despite adequate control of bone turnover [4]. These complications may be due, in part, to alterations in bone mineralization.

Volume

Since PTH is anabolic at the level of trabecular bone, high levels of serum PTH are typically associated with increases in bone volume, trabecular volume, and trabecular width. However, bone volume may also be low (termed “osteoporosis”) particularly in individuals with underlying age-related bone loss or in those treated with corticosteroids. Low bone volume is rare in the pediatric CKD population [41].

Linear Growth

Growth retardation is the hallmark of CKD in children. Growth failure worsens as renal function declines; the average height of children with even mild CKD (GFR 50–70 mL/minute/1.73 m^2) is 1 standard deviation (SD) below the average for healthy children. Moderate CKD (GFR 25 to 49 mL/minute/1.73 m^2) is associated with a height SD of –1.5, and, at the time of initiation of dialysis, the mean height SD is –1.8. Acidosis, calcitriol deficiency, secondary hyperparathyroidism, and adynamic osteodystrophy all may contribute to growth retardation. Treatment for secondary hyperparathyroidism with large, intermittent doses of calcitriol and Ca-based P-binders may decrease linear growth velocity due to dose-dependent inhibitory effects of calcitriol on cell proliferation of chondrocytes and osteoblasts in vitro and to increased expression of IGF-binding proteins [42]. Growth failure persists in many patients despite the correction of the metabolic abnormalities, however, and GH resistance is a major contributor to impaired linear growth in children with even mild kidney disease [43]; further details are discussed in ▶ Chap. 71, “Nutrition, Growth, and Development.”

Interaction Between Primary Kidney Disease and Renal Bone Disease

Rare inherited kidney diseases have recently demonstrated that an element of bone disease may be due to specific mutations – independent of the degree of kidney failure and CKD-MBD – in a subset of children with CKD. Exacerbated skeletal disease is prevalent in patients with primary ciliopathies [44] and those with underlying storage diseases. Ongoing research continues to investigate the interaction between genetics and bone disease in all children with kidney disease.

Autosomal Dominant Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease (ADPKD) is among the most common hereditary nephropathies. Low bone turnover osteopenia characterizes mice with conditional deletion of *PKD1* or *PKD2* genes in osteoblasts, and adults with ADPKD characteristically display low bone turnover, low alkaline phosphatase levels, increased FGF23 levels, and significant hypophosphatemia [45].

Primary Hyperoxaluria

Primary hyperoxaluria is an autosomal recessive defect with a direct deleterious effect on bone. Since bone can store massive amounts of oxalate, patients often present with recurrent low-trauma fractures, bone deformities, and severe bone pain. Calcium oxalate (whewellite) deposits accumulate in the bone marrow space, but not in the bone matrix itself. A granulomatous reaction accompanies this deposition. Overall bone is more brittle than normal [46]. Clinical bone disease worsens in patients treated with dialysis since even intensive hemodialysis regimens remove far less oxalate than is endogenously produced and circulating concentrations of oxalate universally exceed maximum soluble concentrations in patients treated with intensive maintenance dialysis.

Cystinosis

Cystinosis is an autosomal recessive lysosomal storage disease characterized by a deficiency of the cystine lysosomal transport protein (i.e., cystinosin), which results in the systemic accumulation of cystine crystals in many tissues. Cystinotic patients suffer from complete proximal tubulopathy with acidosis and profound hypophosphatemia (Fanconi syndrome) in early childhood; 95% of children have short stature, osteopenia, and rickets. Progressive chronic interstitial nephritis leads to ESKD; secondary hyperparathyroid uremic bone disease thus contributes to bone disease later in the first or second decade of life. In addition, in vitro experiments demonstrate that cystinosin deficiency in bone cells leads to a bone loss phenotype, independent of renal failure. Finally, although cysteamine therapy increases patient and renal survival and decreases systemic comorbidities, cysteamine also affects bone cell function. Low doses of cysteamine have antiresorptive effects and stimulate osteoblastic differentiation and mineralization; by contrast, high doses inhibit osteoblast differentiation in vitro [47]. Thus, the primary disease and its treatment interact with renal P wasting and progressive renal disease to create particularly fragile bone quality in this population (see ► Chap. 37, “Cystinosis”).

Other Genetic Syndromes

Data are limited on the impact of other genetic diseases on bone in children with CKD; however, the role of primary cilia in conditions such as nephronophthisis suggest that patients with mutations in *NPHP6*, *MKS1*, or *MKS3* may have bone pathology that is independent of kidney failure [48]. In addition, the skeletal disease in Pierson syndrome may be exacerbated by the absence of laminin-β2 in bone [49]. In the future, understanding the impact of the different genetic defects on the growing skeleton will likely lead to targeted therapeutic strategies.

Bone Disease After Successful Kidney Transplantation

Successful kidney transplantation corrects many of the bone abnormalities associated with CKD, but disorders of bone and mineral metabolism remain a major problem in the transplant population. Several factors, including persistent secondary hyperparathyroidism, prolonged immobilization, and delayed/poor graft function may negatively affect bone health. Various immunosuppressive agents have also been implicated in the development of bone disease after organ transplantation. Significant bone loss has been shown to occur as early as 3–6 months after kidney transplantation [50].

In both adult and pediatric kidney recipients, iliac crest bone histologic changes associated with secondary hyperparathyroidism resolve within 6 months after kidney transplantation; bone mineral content Z-scores, however, remain significantly reduced, especially in cortical bone [51]. Interestingly, a disconnect is observed between circulating PTH levels and bone turnover posttransplantation; some patients have persistently elevated rates of bone turnover while others develop adynamic lesions, despite moderately elevated serum PTH levels [52]. The use of maintenance corticosteroids may play a causal role; steroids decrease intestinal Ca absorption, enhance urinary Ca excretion, inhibit osteoblastic activity, decrease bone formation, and increase osteoclastic activity and bone resorption [53]. Azathioprine has a minimal impact on skeletal remodeling and the role of other immunosuppressive agents, such as mycophenolate mofetil, as potential modifiers of bone formation and bone resorption have not been evaluated.

After successful kidney transplantation with immunosuppressive regimens standard in many institutions (daily corticosteroids, calcineurin inhibitors, and/or antimetabolites), growth may be accelerated by an improvement in kidney function, but catch-up growth may not be observed even in children who undergo transplantation very early in life [54]. The cause of persistent growth retardation is not completely understood, but immunosuppressive agents, persistent secondary hyperparathyroidism, altered vitamin D and FGF23 metabolism, and the persistence of defective skeletal mineralization may all contribute. Use of rhGH in children with significant height deficit after kidney transplantation may produce a substantial increase in linear growth within the first year of therapy, but the magnitude of growth response may decline thereafter [55]. Calcimimetics may be considered in children with severe persistent hyperparathyroidism, although they may exacerbate low turnover bone disease, even in patients with high serum PTH levels [56].

Evaluation of Renal Bone Disease in Children

Although assessment of bone health is a key element in the clinical care of children with CKD, a paucity of high-quality evidence has led to wide variations in clinical practice in the diagnosis and management of pediatric CKD-MBD. In an effort to standardize care, the European CKD-MBD and dialysis working groups have recently proposed the following clinical practice guidelines for bone evaluation in children with CKD stages 2–5 [57]:

1. Clinical evaluation of height, growth velocity, blood pressure and a musculoskeletal examination, focusing on bone pain, deformities, and fractures, should be performed at baseline and routine intervals. Assessment should be more frequent during periods of rapid growth, such as infancy and adolescence [57]. Ca intake from diet, nutritional supplements, and P-binders should also be assessed at regular intervals (see ▶ Chap. 71, “Nutrition, Growth, and Development”).
2. Traditional biochemical markers of mineral metabolism, including Ca, P, PTH, 25-D, and alkaline phosphatase (ALP), should be measured at routine intervals. Other biomarkers should be measured for research purposes only.
3. Bone imaging, including bone densitometry (DXA or pQCT/HR-pQCT), bone biopsy, and cardiovascular evaluation (coronary artery computed tomography and ultrasound measures of carotid intima/media thickness and pulse wave velocity) are research tools that are not recommended in routine practice [57].

Biomarkers

No perfect biomarker exists for the assessment of all aspects of CKD-MBD; all currently available markers have limited sensitivity or specificity to predict intermediate endpoints like alterations in bone (mineralization and turnover) and vessels (vascular calcifications) or hard end points like fractures or cardiovascular mortality [58]. Thus, a thorough assessment of CKD-MBD parameters requires serial assessment of P, Ca, and PTH levels, all of which should be considered together during clinical decision making [59]. Normal ranges for serum P, Ca, and ALP are age-dependent; ALP values are also gender-specific (Table 1). Monitoring should be based on CKD stage, rate of CKD progression, severity of abnormalities, clinical signs and symptoms, and concomitant medications [57, 60, 61]. Due to the deleterious effects of acidosis on kidney function and on skeletal health, serum bicarbonate levels should also be monitored regularly and maintained within the normal range [57].

PTH directly stimulates osteoblast activity and circulating values of this hormone have been used

Table 1 Age-specific ranges for calcium, phosphate, and alkaline phosphatase

Age range	Calcium (mmol/L)	Ionized calcium (mmol/L)	Phosphate (mmol/L)	25-D (nmol/L)	ALP (IU/L) in boys	ALP (IU/L) in girls
Birth-5 months	2.18–2.83	1.22-1.40	1.50–2.40	75–120	91–476	91–476
6–12 months	2.18–2.75	1.20–1.40	1.50–2.40	75–120	131–476	131–476
1–5 years	2.35–2.70	1.22–1.32	1.50–2.10	75–120	151–342	151–342
6–12 years	2.35–2.58	1.15–1.32	1.20–1.90	75–120	137–424	137–424
13–20 years	2.20–2.55	1.12–1.30	0.70–1.50	75–120	124–474 from 13 to 15	66–262 from 13 to 15
					91-339 from 15 to 17	59-126 from 15 to 17
					64-158 above 17	54-96 above 17

For calcium, the conversion factor from mmol/L to mg/dL is to multiply by 4.0. The calculation formula for corrected calcium (CaC, mmol/L) using measured calcium (mmol/L) and albuminemia (g/L) is the following: $CaC = Ca - 0.25 \times (\text{albuminemia} - 40)$. If albuminemia is not available, CaC may be calculated with protidemia (g/L) with the following formula: $Cac = Ca/(0.55 + P/160)$. For phosphate, the conversion factor from mmol/L to mg/dL is to multiply by 3.1. PTH normal ranges are not presented here since they vary widely with the assay, we propose to reason in terms of x-UNL (x times the upper normal limit for the local assay)

for decades to predict bone turnover in children with all CKD stages [62]. Uncontrolled secondary hyperparathyroidism in CKD is characterized by sustained high PTH levels which contributes to an efflux of Ca and P from bone, together contributing to deleterious effects on the skeleton (osteitis fibrosa), heart, and vasculature (vascular calcifications) [63]. Unfortunately, PTH measurement by all assays is an imperfect predictor of bone turnover and ideal target ranges for PTH remain controversial in all CKD stages [64, 65]. Due to the skeletal PTH resistance that develops as CKD progresses [66], serum PTH levels above the normal range are required to maintain normal rates of bone formation in patients with severe CKD. Two different sets of recommendations related to target PTH levels in dialyzed children exist – the first from the Kidney Disease, Improving Global Outcomes (KDIGO) Working Group and the second from the European Pediatric Dialysis Working Group (EPDWG). Based on bone histology data from the 1990s, which documented more severe growth retardation in patients with PTH < 300 pg/ml along with data from a prospective randomized trial that compared the effects of P-binders and the intermittent administration of vitamin D analogs on the control of the skeletal lesions of secondary hyperparathyroidism, KDIGO guidelines suggest that PTH levels should be maintained between 2- and 9-times the upper limit of normal (i.e., 120–540 pg/mL) [30, 58, 67]. Subsequent to the development of the KDIGO guidelines, new data suggested that high bone turnover lesions can occur at lower PTH levels, particularly in children new to dialysis [68], and data obtained in the International Pediatric Peritoneal Dialysis Registry Network (IPPN) suggested that a range of PTH between 1.7 and 3 times the upper limit of normal (i.e., 100 and 180 pg/mL) was associated with optimal bone quality while higher PTH levels were associated with an increased frequency of bone pain, limb deformities, extraosseous calcifications, and radiographic evidence of osteomalacia or osteopenia [2, 58]. Thus, in 2006, the EPDWG Working Group suggested that PTH levels be maintained between 2- and 3-times the upper limit of normal (120–180 pg/mL) in dialyzed children.

Current data suggest that a combination of biomarkers may be helpful in the management of bone disease in pediatric CKD. In a cross-sectional cohort of 161 pediatric patients undergoing maintenance peritoneal dialysis, PTH levels below 400 pg/ml in combination with total ALP levels below 400 IU/L provided the highest correct prediction rate for patients with both normal bone turnover and normal mineralization [41]. In this study, levels of PTH were higher and serum Ca levels were lower in patients with defective mineralization, irrespective of bone turnover [41]. Thus PTH and alkaline phosphatase trajectories should together guide therapy which should also aim to keep Ca and P levels in the normal range.

In pre-dialysis CKD, data for defining optimal PTH targets are sparse. PTH, P, and FGF23 levels are known to increase well before CKD stage 5 [69]. Concomitantly, bone formation increases in CKD stages 3 and 4 [31]. Data obtained with bone imaging techniques favor strict control of PTH levels; not only do low cortical BMD Z-scores predict subsequent fractures [70] but cortical volumetric bone mineral density is significantly lower in patients with elevated PTH levels than in those with PTH within the normal range [71]. It is important to note that different PTH assays may yield discrepant results, and limited evidence exists that newer assays improve the assessment of bone turnover compared to older assays.

Bone Imaging

Evidence for routine radiological evaluation of bone disease in pediatric CKD is scarce [57]. Severe abnormalities in bone volume and/or mineralization may be observed on conventional radiographs; these films should thus be obtained in children with bone pain, those with suspected atraumatic fractures, and those with genetic diseases with specific bone involvement [72, 73]. Bone radiographs are also useful in patients with clinical manifestations suggestive of avascular necrosis, proximal femoral slipped epiphyses, or in the assessment of skeletal maturity [57]. Skeletal age can be determined on an X-ray of the left wrist and thus predicted adult height can be calculated using the automated Greulich-Pyle bone age determination system. In infants and young children, knee X-rays may be used to evaluate an active metaphyseal area [57]. Extraskeletal calcifications may also be visualized on plane films.

Dual X-ray absorptiometry (DXA), techniques derived from quantitative tomography (pQCT, HR-pQCT), magnetic resonance imaging (MRI), and ultrasound evaluation of bone quality are used in research protocols, but limited evidence exists that DXA (lumbar spine and whole-body) and pQCT results are congruent in pediatric CKD [74] and it remains unclear as to whether DXA scores predict fracture risk in the pediatric CKD population. A recent study showed that combination of routine biomarkers were better predictors of cortical BMD evaluated by pQCT, and BMD measurement by DXA did not correlate with biochemical data or pQCT measures [3]. Thus, these imaging techniques are not recommended for routine screening tools of bone health or fracture risk prediction in pediatric CKD patients [57].

Cardiovascular Disease in CKD

Cardiovascular disease (CVD) is a life-limiting comorbidity in patients with CKD and is rapidly progressive, especially in dialysis patients. The American Heart Association guidelines for cardiovascular risk reduction in high-risk pediatric patients [75] stratify pediatric CKD patients as “high risk” for the development of CVD, with associated “pathological and/or clinical evidence for manifest coronary disease before 30 years of age.”

Epidemiology of Cardiovascular Disease

Data from the USRDS database on over 23,000 children starting dialysis therapy in the United States between 1990 and 2010 demonstrate decreasing rates for overall and cardiovascular mortality in this population [76]. However, data from large registries worldwide report that between 18 and 21% of deaths in children on dialysis are from CV causes [77, 78]. CVD is the primary cause of death in 57% of children receiving HD, 43% of those treated with PD, and 30% of pediatric transplant recipients [79].

Risk Factors for CVD

In addition to traditional “Framingham” risk factors for CVD (i.e., hypertension, dyslipidemia, and smoking) found in the general population, uremia-related risk factors for vascular disease include hypercalcemia, hyperphosphatemia, high PTH, very low or high vitamin D levels, a multitude of middle and large molecular weight substances together labeled “uremic toxins,” anemia, chronic inflammation, oxidative stress, hyperhomocysteinemia, and malnutrition [80]. A number of potential iatrogenic or treatment-related risk factors such as exposure to a high Ca load from dialysate, Ca-based P-binders and vitamin D therapy, advanced glycation end-products, metabolic acidosis, and warfarin therapy can also contribute to the pro-calcific effect of the uremic milieu.

Assessment

“Hard end-points” such as cardiovascular events and death are infrequent in children; thus, surrogate endpoints including left ventricular mass index (LVMI), carotid artery intima-media thickness (cIMT), pulse wave velocity (PWV), and direct evidence of calcification in the coronary arteries and cardiac valves on multi-slice CT scan are used to identify and quantify the burden of cardiovascular disease in pediatric studies. Even small increments in measurements of these parameters are highly predictive of future cardiovascular events in the general population and in adults with CKD. In addition, numerous biomarkers of vascular damage and future cardiovascular events have been described and some validated against “hard end-points” in adult dialysis patients; these include FGF23 [81], the physiological calcification inhibitors (fetuin-A, matrix gla-protein and osteoprotegerin) [82], endothelial microparticles, and cardiac troponin levels [83].

Clinical Studies in Children

A prospective observational study of over 700 - European children with CKD, the Cardiovascular Comorbidity in Children with CKD (4C) study, showed that left ventricular hypertrophy (LVH) was present in 33%, cIMT was elevated in 42%, and PWV was increased in 20% [84]. Similar results were found in reports from the CKiD (Chronic Kidney Disease in Children) study [85].

Although most pediatric studies are small, often single-center, and cross-sectional, remarkably similar associations have been observed between risk factors and cardiovascular disease. A strong linear association has been noted between deteriorating vascular measures and time spent on dialysis [5, 6, 86–89]. Prolonged exposure to the uremic milieu with high, often worsening control of secondary hyperparathyroidism and hyperphosphatemia, exposure to pro-inflammatory agents such as advanced glycation end-products and oxidative stress, and reduced levels of the circulating calcification inhibitors all contribute toward deleterious structural and functional changes in the vasculature. Vascular measures consistently and significantly correlate with circulating Ca, P [86, 87, 89], and PTH levels [6, 86, 89], as well as medication dosages of Ca-based P-binders and vitamin D compounds, suggesting that dysregulated mineral metabolism is central to the vasculopathy of CKD. These modifiable risk factors thus require careful monitoring and strict control from the earliest stages of CKD since increases in cIMT and PWV begin even in the first decade of life in children on dialysis [89] and in those with pre-dialysis CKD [87, 89, 90]. As first demonstrated by Goodman and colleagues, calcifications progress rapidly in patients on dialysis with a doubling of the coronary artery calcification score on CT scan in patients with evidence of calcification on a baseline scan [5]. This suggests that in the pro-calcific and pro-inflammatory uremic milieu “Ca begets Ca” and that primary prevention of calcification, starting in the earliest stages of CKD, is of critical importance.

FGF23 has been linked to cardiovascular disease in patients with all stages of CKD and recent experimental data have defined a direct role for FGF23, independent of changes in mineral metabolism, on pathological changes in cardiac myocytes [91]. Although the effects of circulating FGF23 on mineral metabolism require the presence of Klotho, as the co-receptor of FGF receptors [92], some of the effects on the cardiovascular system appear to be Klotho-independent. FGF23 levels are often hundreds- to thousands-fold higher in patients with kidney disease as compared to individuals with normal kidney function and these values have been associated with an increased prevalence of left ventricular hypertrophy [93] and with premature mortality in adults with all stages of CKD [81]. Recent experimental data have demonstrated that these associations may reflect a direct effect of FGF23 on the myocardium that induces myocyte hypertrophy in a Klotho-independent manner [91]. However, despite the multiple associations between FGF23 and cardiovascular disease, FGF23 levels were not associated with arterial calcifications [94] in a recent large study in patients with CKD stages 2–4.

Pathophysiology of Vascular Calcification

Vascular calcification is an active, highly regulated process and not merely a passive deposition of Ca and P in dead or dying cells [95]. Ca deposition is observed primarily in intimal atherosclerotic plaques in individuals with preserved kidney function, while hydroxyapatite crystals occurs primarily in the tunica media in the context of CKD [96] (Fig. 4). Vascular smooth muscle cells (VSMCs) in the tunica media undergo specific phenotypic changes in the presence of hypercalcemia and hyperphosphatemia; these changes include cellular apoptosis, osteo/chondrocytic differentiation, and the release of small membrane-bound bodies (vesicles) that form a nidus for the deposition of hydroxyapatite [7, 95]. VSMC transformation to an osteo/chondrocytic phenotype is characterized by the upregulation of bone-specific transcription factors and matrix proteins, including RUNX2/CBFA1, osterix, and ALP, that in turn lead to accelerated calcification. Indeed, osteoblasts and VSMCs have a common mesenchymal origin and, in patients with CKD, RUNX2 triggers mesenchymal cell to osteoblast transformation [95, 97].

Elevated serum P concentrations are a key factor triggering osteoblastic differentiation of VSMCs [95], likely mediated in part by expression of the sodium-dependent P transporters PIT-1 and PIT-2 [96]. Upregulation of pro-mineralization factors such as osteopontin, bone sialoprotein, osteonectin, ALP, type I collagen, and bone morphogenic protein-2 (BMP-2) is also potentiated by the uremic milieu [89, 98], while expression of calcification inhibitors, such as fetuin A and matrix gla protein, is suppressed [99]. Alterations in the *WNT* signaling pathway may also play a critical role in the development of vascular calcification [100]. The importance of circulating inhibitors of the calcification process was recently confirmed by several studies using a novel in vitro test (T50 test) to determine the calcification propensity in blood; this quantifies the calcification inhibition of serum that results in the formation of (mainly fetuin-containing) primary calciprotein particles [101].

CKD-MBD Management

Goals of Treatment:

Historically, treatment of CKD-MBD focused on the management of renal osteodystrophy and secondary hyperparathyroidism. More recently, recognizing that serum Ca, P, and PTH levels and vascular calcification are associated with mortality and premature cardiovascular events, treatment goals have focused on preventing premature death and cardiovascular complications. In addition, the high Ca requirement of the growing skeleton must be recognized and appropriately managed to prevent fractures and debilitating bone disease. Principles of management include: maintaining Ca, P, PTH, ALP, and 25D levels within age- and CKD-specific ranges, following trends (rather than single values) of these markers; correcting metabolic abnormalities such as acidosis and malnutrition; promoting optimal growth; and avoiding extraskeletal calcifications. A key objective of CKD-MBD management should focus on primary prevention. Treatments that correct abnormal concentrations of Ca, P, PTH, and vitamin D have demonstrated efficacy across many randomized trials, but high-grade evidence that these therapies affect clinical outcomes such as fractures and cardiovascular

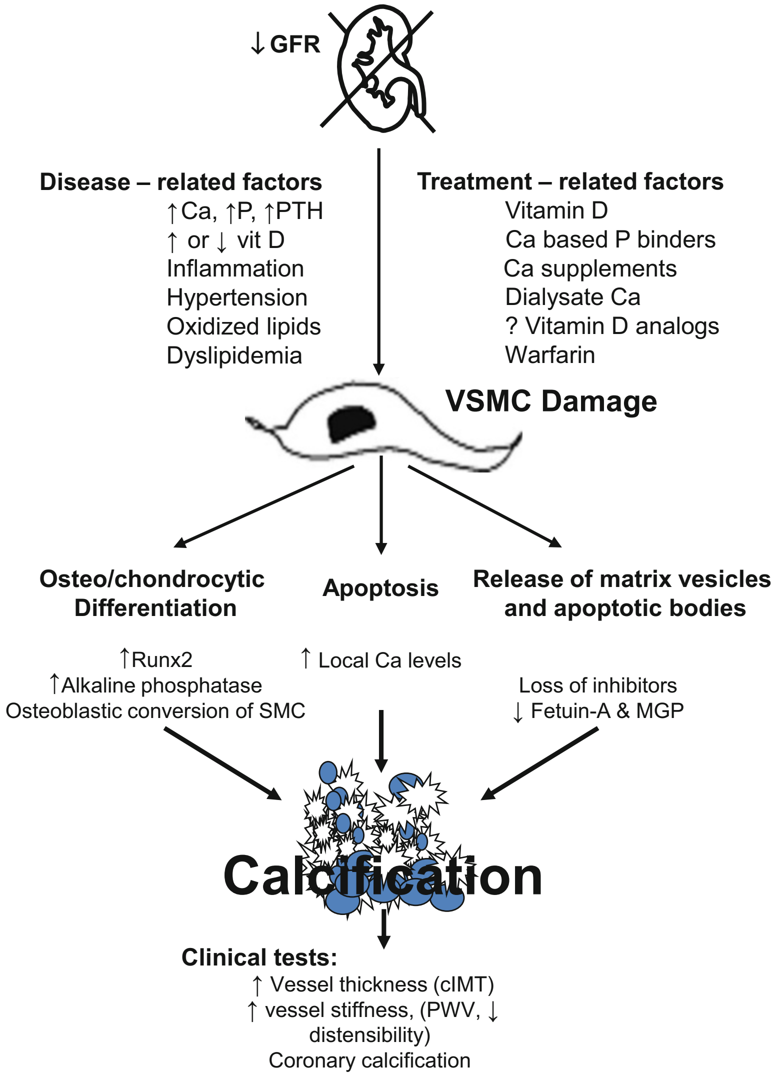
↓GFR
Disease – related factors
↑Ca, ↑P, ↑PTH
↑ or ↓ vit D
Inflammation
Hypertension
Oxidized lipids
Dyslipidemia
Treatment – related factors
Vitamin D
Ca based P binders
Ca supplements
Dialysate Ca
? Vitamin D analogs
Warfarin
VSMC Damage
Osteo/chondrocytic Differentiation
Apoptosis
Release of matrix vesicles and apoptotic bodies
↑Runx2
↑Alkaline phosphatase
Osteoblastic conversion of SMC
↑ Local Ca levels
Loss of inhibitors
↓ Fetuin-A & MGP
Calcification
Clinical tests:
↑ Vessel thickness (cIMT)
↑ vessel stiffness, (PWV, ↓ distensibility)
Coronary calcification

Fig. 4 Model showing the phenotypic changes in vascular smooth muscle cells (VSMCs) in response to mineral dysregulation and uremic insults. In response to injury, VSMCs undergo osteo-chondrocytic changes and convert to bone like cells (osteoblasts). In addition, VSMCs release vesicles that transport the intracellular calcium out of the cell and deposit it in the extracellular matrix, thereby preventing cell death. Extracellular matrix calcification is clinically seen as an increased carotid intima-media thickness (cIMT) and leads to vessel stiffness (increased pulse wave velocity (PWV) and reduced distensibility

events is lacking in children. Extrapolation of findings from studies in adults with CKD may not be appropriate as the growing skeleton of children needs Ca [102], with devastating consequences into adult life if normal bone mineralization has not taken place before peak bone mass is reached [4].

In recent years international guidelines from Kidney Disease Improving Global Outcomes (KDIGO) [30], the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) [103], and the European Society of Pediatric Nephrology (ESPN) [55, 61, 104] as well as many national guidelines such as from the National Institute of Health and Care Excellence (NICE) [105] in the UK have included the management of CKD-MBD in children, and produced clinical practice recommendations on several aspects of CKD-MBD management. As evidence of "hard end-points" is lacking in children, many recommendations are "weak statements" or are opinion based only and must be carefully considered and adapted to individual patient needs based on the clinical judgment of the treating physician.

Dietary Management of CKD-MBD

Regular dietary counseling is an essential part of CKD-MBD management and is discussed in detail in ▶ Chap. 71, “Nutrition, Growth, and Development.” As high FGF23 levels are observed before abnormalities in serum Ca, P, or PTH develop, optimal control of serum P concentration is a crucial element of preventative management. K/DOQI [103] and the Pediatric Renal Nutrition Taskforce [106], have published guidelines on the dietary management of Ca and P in children with CKD2-5D. Both groups recommend regular dietary counseling from early CKD, limiting dietary P intake to within the age-appropriate normal ranges for children in the mild-moderate stages of CKD and to the lower limit of the normal range (K/DOQI suggests 80% of dietary recommended intake) in patients with advanced CKD who have persistent hyperphosphatemia or hyperparathyroidism [106, 107].

The average P intake of both adults and children on a “western diet” is approximately 1500–2000 mg/day, and 60–70% of the dietary intake is absorbed. Importantly, processed foods are likely to contain inorganic P additives (such as phosphoric acid and sodium phosphate), which are almost completely absorbed, unlike organic phosphates that have a lower bioavailability. Additives may increase food P content by 60–70 mg per 100 g of product [108]. Processed foods with significant amounts of added inorganic P include meat, dairy and bakery products, and drinks such as colas; their presence but not their quantity is indicated as an “E number.” In addition, inorganic phosphates are added to several medications, such as antacids and many anti-hypertensive medications as an excipient, and can significantly increase the daily P intake.

Of note, aggressive dietary P restriction is difficult since it may compromise adequate intake of other nutrients, especially protein and Ca [109]. P intake is directly linked to protein intake, with 10–12 mg of P accompanying each gram of protein; any reduction in P intake should not compromise protein intake. In a recent study 67% of children with CKD stages 4–5 had a dietary Ca intake below the recommended nutrient intake, and additional Ca from medications, mainly in the form of P binders, was required to maintain nutritional Ca requirements [109].

Phosphate Binders

As renal function deteriorates, dietary P control becomes more difficult and overt hyperphosphatemia often develops. Phosphate binders (P-binders) reduce intestinal P absorption by forming poorly soluble complexes with P in the intestinal tract; these binders are of two main types: Ca-based and Ca-free (Table 2). It remains uncertain when along the spectrum of CKD P-binders should be initiated, what level of serum P should be targeted to improve outcomes, and whether all P-binders are equivalent in terms of long-term outcomes. No evidence exists that specific target levels of serum P improve clinical endpoints. In a randomized clinical trial in adults with an eGFR of 20–45 ml/min/1.73 m^2, increases in coronary calcification were noted in patients with normal serum P levels who were treated with Ca, lanthanum, and/or sevelamer binder therapy, but not in placebo treated patients [110]. Similar data are not available for children.

Ca-Based P-binders

KDOQI recommendations for the treatment of children with CKD stages 2–5 suggest that the choice of P-binders therapy should be guided by serum Ca levels [111]. Ca-based P-binders should be used as first-line therapy except in those with overt hypercalcemia [105]. Several Ca salts, including Ca carbonate, Ca acetate, and Ca citrate, are commercially available. Ca carbonate is the most commonly used compound, and studies in adults and children have shown its efficacy in controlling serum P concentrations [63, 68, 112]. Ca acetate contains 25% of elemental Ca (in contrast to Ca carbonate which contains 40%) and is a more effective binder per unit Ca content than is Ca carbonate (Table 2). Ca citrate and ketoglutarate are also effective P-binders, but gastrointestinal side effects and high cost limit their use. Due to the stimulatory effects of Ca on

Table 2 Phosphate binders

Compound	Formulation(s) available	Calcium Content (%)	Calcium Absorbed (%)	Phosphate Bound per mg Ca^{++} Absorbed	Comments
Calcium carbonate	250 mg, 500 mg, 1.25 g, 2.5 g tablets or as 250 mg/5 ml liquid	40	20–30	≈ 1 mg/ 8 mg	High Ca load, cheap, few GI side effects
Calcium acetate	475 mg or 950 mg tablets	25	22	≈ 1 mg/ 3 mg	Less Ca load than $CaCO_{3,}$ inexpensive, GI side effects more common particularly in infants
Sevelamer hydrochloride or sevelamer carbonate	800 mg tablets for both or 2400 mg sachet (for sevelamer carbonate only)	0	0	NA	Ca^{++} and Al^{+++} free, acts as cholesterol-binding resin and lowers serum cholesterol, binds fat soluble vitamins, expensive, difficult to administer to younger children, metabolic acidosis (with hydrochloride preparation)
Aluminum containing binders	100 mg capsules	0	0	NA	Very effective P-binding but risk of aluminum toxicity; can be used short term under close monitoring of aluminum levels; recommended for "rescue therapy" from severe hyperphosphatemia only
Mg + Ca carbonate	Variable strength tablets	Variable	20–30% of Ca	≈ 1 mg/ 2.3 mg	Less Ca^{++} load, GI side effects, long-term effects?
Lanthanum carbonate	Chewable tablets or sachets with 500 mg, 750 mg, or 1000 mg	0	0	NA	Poorly tolerated as gastrointestinal side effects are very common; accumulates in bone and long-term effects in the growing bone are unknown; expensive
Sucro-ferric oxyhydrixyde	500 mg chewable tablets	0	0	NA	Gastrointestinal side effects – diarrhea, nausea

NA not applicable

FGF23 expression [113], Ca-based P-binders appear to be less effective in lowering FGF23 levels than do non-Ca binders such as lanthanum and sevelamer [114–116]. Table 3 provides practical tips on the use of P-binders and vitamin D medications.

Ca-Free P-binders

Large doses of Ca carbonate may lead to hypercalcemia, particularly in patients treated with vitamin D or those with adynamic bone disease [63] and adult pre-dialysis CKD patients have been shown to be in a positive Ca balance when receiving as little as 1500 mg/day of Ca carbonate [117]. Current KDIGO recommendations do not specify maximum doses of Ca-based binders, but acknowledge the potential existence of a safe upper limit of Ca dose [111]. While the growing skeleton has a higher demand for Ca, Ca-free P-binders are indicated in patients with hypercalcemia, either alone or in combination with Ca-based binders. Table 2 lists the commonly used non-Ca-based binders, their P-binding capability, and their side effects.

– ***Sevelamer Hydrochloride***, a Ca- and aluminum-free hydrogel of cross-linked polyallylamine, lowers serum P without inducing hypercalcemia in adult patients treated with hemodialysis [118]. Because hydrochloric

Table 3 Practical tips on the use of phosphate binders and vitamin D medications

Phosphate Binders	Vitamin D
Children and their caregivers benefit from regular diet education sessions with help from renal dietitians, as well as doctors and nurses, to understand the complexities of their renal diet	Serum concentrations of 25D are the best marker of the vitamin D status of an individual
	Frequency of measurement of 25D levels should not be more than 3-monthly
	$1{,}25(OH)_2D$ should not be measured for routine assessment of vitamin D status
Should be given with meals and high P snacks, never on an empty stomach	Ergocalciferol or cholecalciferol may be used interchangeably if daily, weekly, or monthly treatment regimens are used
Significantly more Ca is absorbed when Ca containing P-binders are not given with meals	
Do not give a fixed dose, but adjust the dose depending on the P content of the meal or snack	All vitamin D analogs are adsorbed to plastic tubing to varying degrees, and therefore should not be given via feeding tubes
Calcium carbonate requires an acidic pH in the stomach to dissociate and bind P. Hence, calcium carbonate must not be given along with an antacid or H2-antagonist or sodium bicarbonate	Vitamin D analogs increases intestinal P absorption from around 60–90%; calcitriol use may be limited by worsening hyperphosphatemia and hypercalcemia
Calcium will precipitate in the duodenum as calcium carbonate if given with sodium bicarbonate medications	A vitamin D treatment regimen guided by age and baseline vitamin D concentration is recommended
Restriction of dairy foods as part of a P controlled diet limits Ca intake. Additional Ca from medications is required to meet the KDOQI guideline of 100–200% normal recommended Ca intake	An intensive replacement phase (usually for 3 months) followed by a maintenance phase is recommended by most guidelines

acid is released when P binds, this medication may induce a metabolic acidosis. The use of sevelamer carbonate circumvents this side effect [119]. Two randomized controlled studies in children have demonstrated that sevelamer hydrochloride has an equivalent P-binding capacity as Ca acetate, and does not increase serum Ca levels [120, 121]. In addition to its effects on serum P concentrations, sevelamer decreases concentrations of total serum cholesterol, decreases low-density lipoprotein cholesterol, and increases high-density lipoprotein levels [122].

- ***Aluminum Hydroxide*** is a very efficient P-binder, but its long-term treatment can lead to adynamic bone disease, encephalopathy, and anemia [123], hence aluminum may only be used as a short-term "rescue" treatment when severe hyperphosphatemia is present associated with hypercalcemia that is not responsive to other P-binders [63, 112]. If this drug is used, regular monitoring of aluminum blood levels is essential.
- ***Lanthanum Carbonate*** binds intestinal P more effectively than Ca carbonate and sevelamer. However, patients on extended treatment show increased lanthanum serum levels. Lanthanum also accumulates in the bone in dialysis patients, where its presence persists despite discontinuation for as long as 2 years [124]. In light of the experience with aluminum toxicity, lanthanum containing P-binders are currently not recommended in children with CKD. Additional long-term studies are needed to confirm the absence of toxicity before this agent is recommended for widespread use.
- ***Iron-Based P-Binders*** reduce serum P and FGF23 concentrations while simultaneously increasing serum iron parameters [125, 126]. A recent randomized open-label study has shown that sucroferric oxyhydroxyde is as effective as Ca acetate in controlling serum P levels in children with CKD [126]. Similar to other P-binders, adverse events are primarily GI-related.
- ***Magnesium Salts*** have a relatively weak intestinal P-binding activity. Diarrhea, hyperkalemia, and hypermagnesemia are their main side effects and the long-term effects of an increased magnesium load are not clear.

Magnesium-free dialysate solutions should be used in patients treated with maintenance dialysis.

- ***Newer P-Binders***: Intestinal P absorption is regulated by sodium-dependent P co-transporter type 2b (NPT2b), therefore treatment with niacin (vitamin B3) or its metabolite nicotinamide/niacinamide, both of which modulate NPT2b expression, result in a sustained reduction in serum P concentrations in adult dialysis patients [127]. However, in a randomized trial of adult HD patients, NPT2b inhibition was not effective in reducing serum P concentrations [128]. Targeting salivary P using P-binding chewing gum has also been proposed [129]. These agents have not been studied in children.

Vitamin D Treatment

The K/DOQI [103], recent KDIGO update [111] and the ESPN [61, 130] have all developed guidelines on the management of vitamin D deficiency and use of active vitamin D analogs in children with CKD stages 2–5. Native vitamin D therapy is indicated in children with pre-dialysis CKD and those treated with maintenance dialysis who have evidence of vitamin D deficiency or insufficiency while active vitamin D therapy is indicated for the treatment of secondary hyperparathyroidism despite normal 25D levels, provided there is no hypercalcemia and/or hyperphosphatemia. Table 3 provides practical tips on the use of vitamin D medications.

Native (Nutritional) Vitamin D Supplementation

All guidelines suggest that serum 25D levels are routinely measured in CKD patients [61], although target ranges vary between guidelines. The ESPN guidelines suggest that serum 25D levels be maintained above 75 nmol/L (30 ng/ml), and below 120 nmol/L (48 ng/ml) [61]. These levels maintain optimal gut Ca absorption and prevent seasonal fluctuations that can predispose even healthy children to nutritional rickets [131]. In the only RCT of 25D therapy in children with CKD stages 2–4, children on ergocalciferol who achieved 25D levels >75 nMol/L had a significantly longer time to development of secondary hyperparathyroidism compared to those on placebo [10]. No clear consensus exists between guidelines on the type of native vitamin D supplement (ergo vs cholecalciferol), its dosage, frequency of administration, or duration of treatment in healthy children or children with CKD [131]. All guidelines recommend a loading regimen or intensive replacement period for a variable duration of 4–12 weeks followed by a maintenance regimen [61, 103]. K/DOQI and the ESPN guidelines recommend escalating doses for intensive replacement depending on the baseline 25D level [61, 103]; cholecalciferol doses range 2000–8000 IU/day for intensive phase replacement and 1000–2000 IU/day for maintenance therapy. Fortunately, vitamin D has a wide therapeutic window and hypercalcemia, hypercalciuria, and symptomatic toxicity has been reported only when serum 25D levels exceed 250 nmol/L.

Active Vitamin D Sterols

Treatment with active vitamin D sterols is indicated in pediatric CKD patients whose serum PTH levels remain elevated despite normal serum 25D and P levels. Alfacalcidol (1-α hydroxyvitamin D_2) or calcitriol (1,25-dihydroxyvitamin D_3) are most commonly used. An initial dose of 5–10 ng/kg/day is effective and safe in most children with CKD; the lowest dose that maintains normal serum PTH levels should be used.

With prolonged treatment with vitamin D analogs, children may develop adynamic bone disease, which has been associated with a reduced growth rate and frequent episodes of hypercalcemia [132]. Calcitriol usage may contribute to extraosseous calcifications in children with ESKD [5–7, 133]. Synthetic vitamin D analogs have been developed to reduce intestinal Ca and P absorption at equipotent PTH suppressive action. Three sterols have been approved – 22-oxacalcitriol (maxacalcitol) in Japan and 19-nor-1,25-$(OH)_2D_2$ (paricalcitol) and 1 α-(OH) D_2 (doxercalciferol) in the United States. A comparative trial of calcitriol and doxercalciferol in pediatric dialysis patients revealed no differences in the control of secondary hyperparathyroidism, development of hypercalcemia, or increase in FGF23 levels [38]. Although both agents suppressed bone turnover, neither were able to normalize histomorphometric parameters of skeletal mineralization and a mineralization defect remained in the majority of patients. Two randomized trials have demonstrated that vitamin D analogs do not improve cardiac function for patients with CKD stages 3–5 during 1 year of treatment [134, 135].

All activated vitamin D analogs increase FGF23 secretion. Although the consequences of these increased FGF23 levels in dialyzed patients remain to be completely determined, current evidence suggests that excessive circulating FGF23 is associated with increased mortality rates and cardiovascular disease [81]; however, prospective randomized trials are required.

Calcimimetic Agents

Cinacalcet is an allosteric modulator of the Ca-sensing receptor (CaSR), which is expressed in several tissues, including the parathyroid glands. Cinacalcet enhances the CaSR sensitivity for extracellular Ca, reducing serum PTH, Ca, and P concentration for improved control of secondary hyperparathyroidism [136]. The largest RCT evaluating calcimimetic use, the Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events (EVOLVE) trial included 3883 adult patients on HD with baseline PTH levels of ≥300 pg/ml (31.8 pmol/l) and Ca concentration ≥8.4 mg/dl (2.1 mmol/l) [137]. Patients receiving cinacalcet were less likely to reach the primary composite end-point of time to death, myocardial infarction or hospitalization for a cardiovascular event (hazard ratio 0.88 (95 CI: 0.79-0.97)) [137]. Secondary analyses suggested benefit from cinacalcet for non-atherosclerotic cardiovascular events (like admission for heart failure) and a lower incidence of parathyroidectomy. A meta-analysis that included 23 RCTs with a total of 8481 adult dialysis patients failed to demonstrate a significant reduction in the risk of fractures, cardiovascular events, or mortality with cinacalcet treatment, despite a significant decrease in PTH and decreased incidence of parathyroidectomy [138].

Five industry-sponsored RCTs or open label trials have evaluated cinacalcet use in children on dialysis; of these, only two are published [139, 140]. Enrolled patients had baseline intact PTH levels above 300 pg/ml (6-fold upper limit of normal [ULN]) and Ca levels above 2.2 mmol/l; the primary end-point of at least a 30%-reduction in PTH from baseline was achieved in one study [139]. Five other prospective studies have reported that cinacalcet is effective in reducing PTH levels in children with baseline PTH values of 300–500 pg/ml (9-fold ULN), but report varying rates of hypocalcemic episodes after cinacalcet exposure.

A recent ESPN consensus statement [104] recommends the use of cinacalcet in dialyzed children older than 3 years of age who have persistent and severe hyperparathyroidism in the presence of high or high-normal Ca levels, despite optimized conventional management (including vitamin D sterols). Importantly, because cinacalcet can induce hypocalcemia, leading to potentially fatal arrhythmias or seizures, cinacalcet should not be started in patients with albumin-corrected Ca levels below 2.40 mmol/L. Cinacalcet must be carefully titrated against serum Ca and PTH, using the minimal effective cinacalcet dose used to maintain PTH levels in the desired target range. A fatal adverse event was reported in an adolescent girl with prolonged QT treated with cinacalcet [139]; thus, cinacalcet should not be started in patients with a prolonged QT interval and should be used with caution in patients with a history of seizures, cardiac arrhythmia, significant liver disease, and in those on medications that may increase the QTc or interfere with cinacalcet metabolism. Regular monitoring of serum Ca (ionized Ca, if available) and PTH levels is essential and conventional therapy, including nutritional Ca intake, Ca-based P-binders, vitamin D analogs, and dialysate Ca, may need to be adjusted to achieve normal circulating Ca, P, and PTH concentrations.

A newer calcimimetic, etelcalcetide, is a synthetic peptide that allosterically activates the CaSR in the extracellular domain of the receptor, located on the parathyroid chief cells. By binding to the CaSR, etelcalcitide enhances the activation of the receptor by extracellular Ca, which results in decreased PTH secretion [141]. This mechanism contrasts with cinacalcet, which directly lowers PTH by increasing the sensitivity of the CaSR to extracellular Ca [136]. Etelcalcitide is administered intravenously after each HD session, potentially improving patient compliance. Studies in adults suggest that etelcalcetide decreases serum PTH levels to a similar extent as cinacalcet [142]; studies in children are ongoing.

Surgical Parathyroidectomy

With the availability of calcimimetics, the incidence of unremitting hyperparathyroidism and the need of parathyroidectomy has decreased [143]. However, in patients with contraindications or incomplete responses to calcimimetics, parathyroidectomy is still indicated in the context of persistent and recurrent hypercalcemia and hyperphosphatemia despite optimized medical management, progressive and debilitating bone disease, progressive extraosseous calcifications, and/or calciphylaxis [144]. Of note, other causes of hypercalcemia and bone disease must be excluded and parathyroid gland imaging by ultrasound or radionuclide studies should be used to identify the presence of parathyroid hyperplasia, hypertrophy, or adenomas that render a poor response to medical therapy. To avoid profound and life-long risk of hypocalcemia, autotransplantation of parathyroid tissue fragments in the abdominal subcutaneous layer may be an alternative to a total parathyroidectomy; the autotransplanted parathyroid tissues tend to grow and secret PTH.

Antiresorptive Therapies

Bisphosphonates and receptor activator of nuclear factor-κB ligand (RANKL) inhibitors (denusomab) are widely used for the treatment and/or prevention of osteoporosis and its complications in the general population. Bisphosphonates are eliminated via renal excretion and may cause an acute kidney injury that is not always fully reversible; their use even in adults with CKD is limited [145]. In pediatric CKD patients, the efficacy and safety of bisphosphonates have not been studied and only case reports of bisphosphonate use, mainly in the context of treating hypercalcemia or after renal transplantation have been reported [146]. Careful morphological assessment of bone structure and formation is required as bisphosphonates, via a deceleration of bone turnover, have the potential not only to aggravate preexisting adynamic bone disease, but also to impair longitudinal growth [147].

Denusomab is a human monoclonal antibody that binds and inhibits the actions of receptor activator of nuclear factor-κB ligand (RANKL), which is expressed by osteoblastic cells and mediates osteclastic bone resorption. Denusomab increases BMD and prevents fracture [148] in older women with low bone density. Preliminary studies indicate that the pharmacokinetics of denusomab is not affected by kidney function [148]. Its effectiveness has not been studied in children with CKD, and, of note, the risk of symptomatic hypocalcemia in children may be even more significant than in adults.

Growth Hormone Therapy

A recent guideline from the ESPN recommends that children in CKD stages 3–5 should be candidates for growth hormone therapy if they have persistent growth failure after other potentially treatable risk factors for growth failure have been adequately addressed, and provided they have growth potential [55]. Growth hormone therapy is discussed further in ▶ Chap. 71, “Nutrition, Growth, and Development.”

Dialysis

Standard prescriptions of peritoneal dialysis and hemodialysis remove insufficient amounts of P; 300–400 mg/day are removed daily with peritoneal dialysis and 800 mg in a standard hemodialysis session. By contrast, the use of daily, slow, continuous hemodialysis achieves excellent P removal, often allowing P-binders to be discontinued.

Indeed, some patients have developed hypophosphatemia and have required the addition of P to the dialysate solution to prevent the long-term consequences of hypophosphatemia [149, 150]. In the Frequent Hemodialysis Network (FHN) Daily Trial, dialysis six times per week lowered serum P by 0.18 mmol/l compared with dialysis three times per week [151]. Similarly, frequent nocturnal hemodialysis lowered serum P levels by 0.45 mmol/l compared with conventional hemodialysis. Higher dialysate Ca levels (1.5–1.75 mMol/L) are required in children on long daily or nocturnal HD; this is discussed in the chapter on hemodialysis (see Chapter 77).

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Anemia in Chronic Kidney Disease

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Abstract

Anemia is common in children with chronic kidney disease (CKD), and the prevalence of this comorbidity increases with advancing CKD stage, affecting nearly all children treated with chronic dialysis. The primary causes of anemia in CKD are erythropoietin (EPO) deficiency/dysregulation, and iron deficiency/disorders of iron metabolism. This chapter reviews the pathogenesis of anemia and current approaches to clinical management including the initial diagnostic evaluation and the use of erythropoiesis stimulating agents (ESA) and iron supplementation. Clinical practice guidelines for anemia treatment goals are reviewed. Particular attention is paid to emerging novel anemia therapies made possible by our improved understanding of the molecular regulation of both EPO production and iron metabolism in the setting of CKD; longer-acting ESA, hypoxia inducible factor stabilizers/prolyl hydroxylase inhibitors, and novel approaches to iron supplementation and management of hepcidin-mediated iron restriction. The approach to ESA-resistant anemia is also discussed. Finally, the approach to prevention, evaluation, and management of anemia after successful kidney transplantation is discussed.

Keywords

Hemoglobin · Erythropoiesis stimulating agent · Iron · Hepcidin · Ferroportin · Transplant

Synonyms

Anaemia; End Stage Kidney Disease; ESA

Introduction

The association between renal failure and anemia was first noted nearly two centuries ago, when the failure of erythropoiesis was described as “close to hemorrhage for impoverishing the red particles of the blood” [1]. The prevalence of anemia increases with advancing chronic kidney disease (CKD) stage, and affects nearly all children treated with chronic dialysis. Data from the Chronic Kidney Disease in Children (CKiD) cohort study have shown that median hemoglobin levels decline as the measured glomerular filtration rate (GFR) decreases below a level of 43 ml/min/1.73m^2 [2]. The emergence of recombinant human erythropoietin (rHuEPO) therapy more than 30 years ago revolutionized anemia management in patients with kidney disease and eliminated dependence on red blood cell transfusions for most patients. To date, erythropoiesis stimulating agents (ESA) and iron supplementation have been the standard of care to treat the anemia of CKD. However, anemia management remains challenging and treatment resistance is not uncommon. An improved understanding of the molecular regulation of both EPO production and iron metabolism has opened the door for the development of novel renal anemia therapies. This chapter reviews current approaches to the clinical management of the anemia of CKD, with focus on the mitigation of the primary causes of the anemia of CKD; EPO deficiency and dysregulation; and disorders of iron metabolism.

Pathogenesis of Anemia in CKD

Normal Erythropoiesis

Normal erythropoiesis becomes disordered in CKD. Erythrocytes lost to senescence and bleeding must be continually replaced. Erythropoiesis consists of the generation of mature red cells from multipotential myeloid stem cells and includes two distinct phases; an initial EPO-dependent phase which includes the proliferation and maturation of erythroid precursors, and a second phase of differentiation from erythroblasts to reticulocytes which is strongly iron-dependent [3, 4]. (Fig. 1) The glycoprotein hormone EPO is the 30.4-kDa product of the *EPO* gene on chromosome 7 [5, 6]. Prenatally, the liver is the primary site of EPO production, but this shifts to the kidney after birth, with a small additional amount continually produced by the liver [5]. EPO is produced by the interstitial fibroblast-like cells in the peritubular capillary bed of the renal cortex [5]. When injured, these cells transdifferentiate into myofibroblasts which synthesize collagen, losing the ability to produce EPO [5].

Hypoxia Stimulates Red Blood Cell Production

The cellular sensing of tissue hypoxia is the key signal leading to upregulation of EPO production, via EPO gene transcription through the actions of hypoxia inducible factor (HIF). The HIFs are transcription regulators which control the rate of gene transcription by binding to specific DNA sequences, and thus are key mediators of cellular adaptation to oxygen deprivation [4]. HIF comprises an oxygen sensitive α-subunit (HIF-1α, HIF-2α, or HIF-3α) and a stable β-subunit, and activates the transcription of genes containing hypoxia response elements, including the *EPO* gene

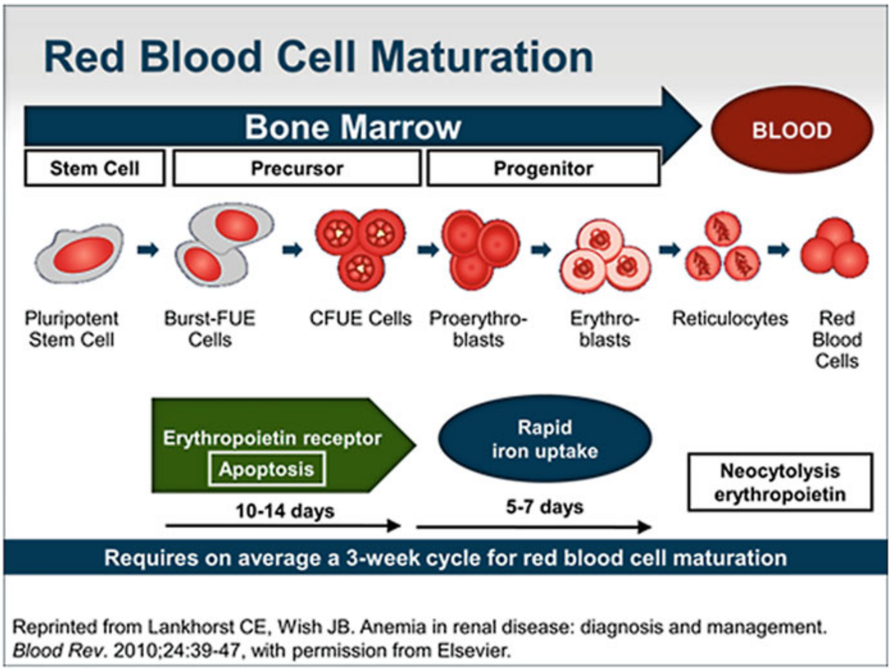
Red Blood Cell Maturation
Bone Marrow
BLOOD
Stem Cell
Precursor
Progenitor
Pluripotent Stem Cell
Burst-FUE Cells
CFUE Cells
Proerythro-blasts
Erythro-blasts
Reticulocytes
Red Blood Cells
Erythropoietin receptor
Apoptosis
10-14 days
Rapid iron uptake
5-7 days
Neocytolysis erythropoietin
Requires on average a 3-week cycle for red blood cell maturation

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Fig. 1 Red blood cell maturation cycle [66]. (Reprinted from Ref. [66], with permission from Elsevier)

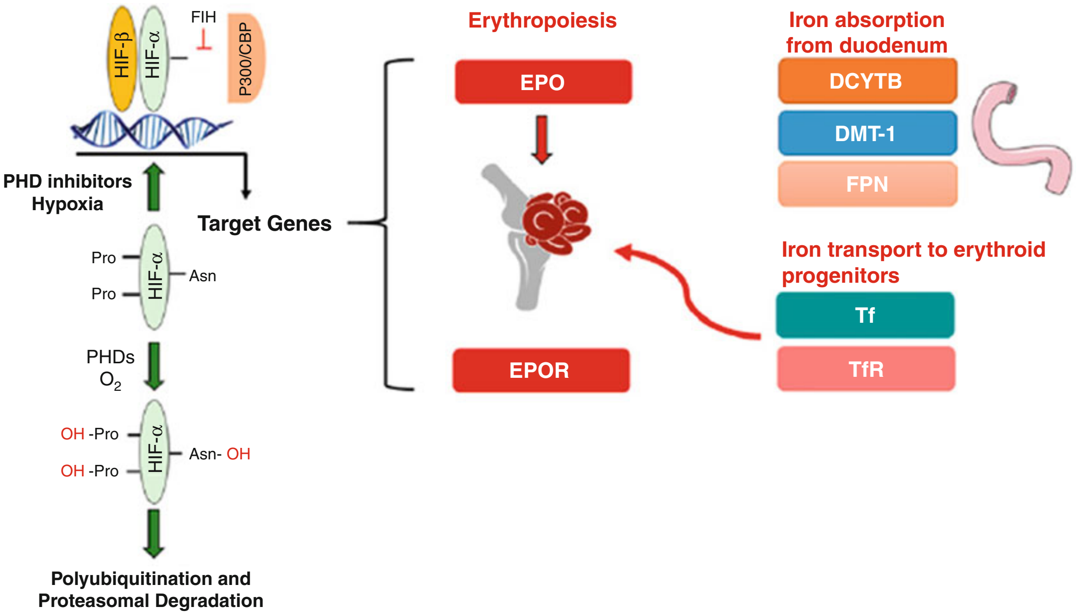

Fig. 2 Role of hypoxia-inducible factor α (HIF-α) in regulation of erythropoiesis [4]. (Reprinted with permission). Hypoxia signaling controls erythropoiesis by coordinating EPO synthesis with the expression of genes involved in iron metabolism. Under well-oxygenated conditions, the three oxygen-labile HIF-*α* subunits (HIF-1*α*, HIF-2*α*, and HIF-3*α*) are hydroxylated at specific proline (Pro) residues by PHD engines. Prolyl hydroxylation targets HIF-*α* proteins for ubiquitination by the von Hippel-Lindau (pVHL)-E3-ubiquitin ligase complex with subsequent proteasomal degradation. Under conditions of reduced PHD activity (e.g., hypoxia or pharmacologic inhibition), HIF-*α* escapes hydroxylation and translocates to the nucleus, where it forms a heterodimer with the constitutively expressed HIF-*β* subunit. The effective HIF-*α*/HIF-*β* complex activates the transcription genes whose promoters contain hypoxia response elements. The HIF-mediated activation of transcription requires the recruitment of coactivators such as p300/CREB-binding protein. An additional layer of regulation is due to factor-inhibiting HIF (FIH), which hydroxylates a specific asparagine (Asn) residue abrogating transcriptional cofactor recruitment. HIF-*α* stabilization results in the activation of genes in diverse biologic processes. For instance, HIF-2*α* induces EPO production from renal peritubular fibroblasts and hepatocytes, promoting erythroid progenitors' cell viability, proliferation, and differentiation through EPO receptor (EPOR) signaling. Genes expressed in duodenum, *duodenal cytochrome b reductase (DCYTB)* and *divalent metal transporter-1 (DMT1)*, along with *ferroportin* (*FPN*) are activated by HIF-2, increasing iron uptake while HIF signaling also controls the expression of iron transport genes *transferrin* (*Tf*) and *transferrin receptor* (*TfR*). O_2 oxygen; OH, hydroxide

[4, 7]. HIF-α proteins are continually produced, but in the presence of normoxia are "marked" (hydroxylated) for degradation by the HIF-prolyl hydroxylases [1]. (Fig. 2) Once hydroxylated at proline residues, HIF-α is targeted for ubiquitination and proteasomal degradation by the von Hippel-Lindau protein [1, 4]. Under hypoxic conditions, HIF-α is stabilized and, after nuclear translocation, dimerizes with the HIF-β subunit forming heterodimers that activate the *EPO* gene and other genes associated with iron metabolism [1, 4]. (Fig. 2) Notably, HIF-2α induces EPO production from both renal peritubular fibroblasts and from hepatocytes [4]. HIF-prolyl hydroxylase inhibitors have emerged as a promising class of therapeutic agents for the treatment of the anemia of CKD, as will be discussed later in this chapter.

Dysregulated Erythropoiesis in CKD

Decreased EPO production by injured fibroblast-like epithelial cells is not the only means by which endogenous EPO production is decreased in kidney disease. Diminished oxygen consumption by diseased renal tissue leads to dysregulation of EPO production by increasing tissue oxygen pressure, which in turn leads to decreased HIF stability

[8]. The result is decreased EPO transcription occurring independently of damage to EPO-producing cells.

Iron Is Required for the Synthesis of Hemoglobin

Effective erythropoiesis depends not only on erythropoietin production, but also on the availability of iron, and incorporation of iron into erythroblasts is a rate-limiting step in the maturation of red blood cells in the bone marrow. Because iron does not have an excretion regulation pathway, dietary intake, intestinal absorption, and iron recycling must be finely regulated at multiple stages of the red blood cell life cycle [3, 4]. (Fig. 3) The majority of this iron is supplied by macrophages which recycle iron from senescent red blood cells, while only 1–2 mg per day of iron comes from intestinal absorption [3]. Transferrin is the glycoprotein iron transporter that binds tightly but reversibly to iron in plasma, preventing the oxidative stress that freely circulating iron would induce. Iron that has been transported into the circulation bound to transferrin is released to erythroblasts via the interaction of transferrin with the transferrin receptor and receptor-mediated endocytosis [3].

Hepcidin Regulates the Ferroportin-Based Movement of Iron

The iron-regulatory protein hepcidin has emerged as the key regulator of iron homeostasis [9]. Hepcidin is produced in hepatocytes and regulates both intestinal iron absorption and body

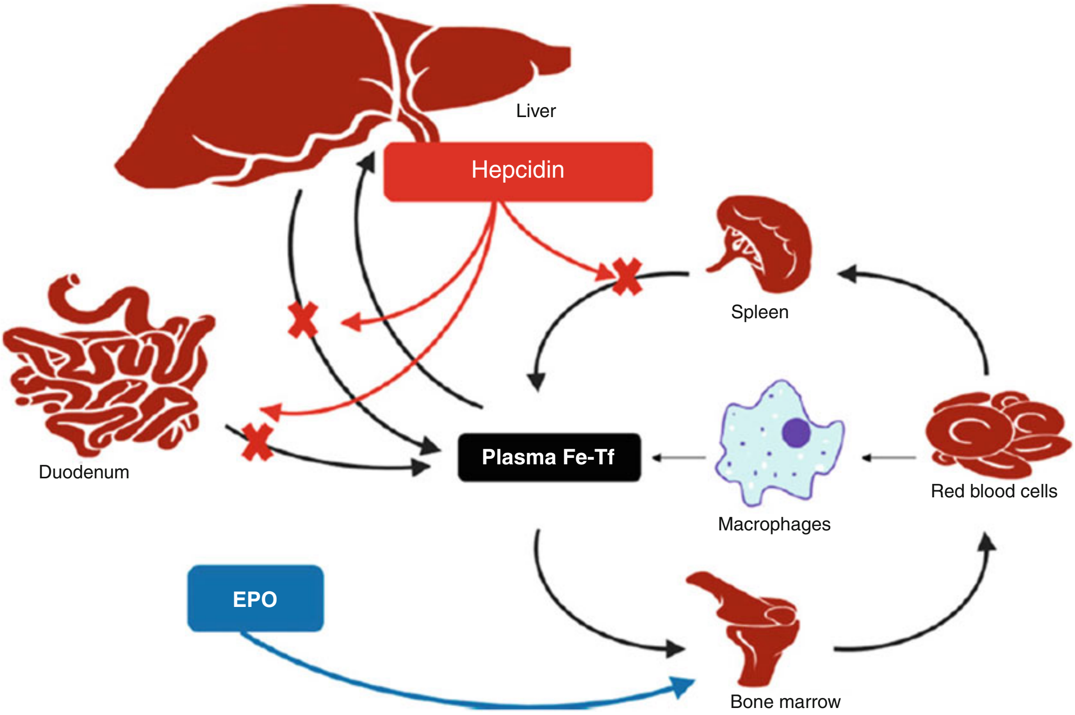

Fig. 3 Iron metabolism is a tightly controlled process [4]. (Reprinted with permission). Iron metabolism is a tightly regulated process. Iron is absorbed in the gut and bound to soluble transferrin. Iron is then moved to storage in the bone marrow and used for erythropoiesis. Additional stores are repleted by macrophage uptake of iron from RBC destruction. EPO induces RBC production, leading to the mobilization of iron stores from the bone marrow. Hepcidin, which is produced by the liver and often stimulated by inflammation, leads to decreased iron uptake from the gut and decreased mobilization of iron stores. Fe-Tf, iron-bound transferrin

iron distribution through its post-translational suppression of cell membrane expression of ferroportin, which is the sole cellular iron exporter. Hepcidin binding to ferroportin causes internalization and lysosomal degradation of ferroportin, which results in downregulation of dietary iron absorption via intestinal enterocytes, and inhibits the release of stored iron from reticuloendothelial cells [10]. In this way, hepcidin prevents the utilization of absorbed or stored iron for erythropoiesis by the bone marrow. Hepcidin expression is induced by inflammation in general and in particular by the inflammatory cytokine IL-6. Hepcidin is cleared via the kidney, leading to increased levels in the setting of decreased GFR [11]. Hepcidin is also cleared from the circulation by hemodialysis [12]. Direct modulation of hepcidin production may be a promising future avenue for treatment of iron restriction in CKD, and current investigational strategies include monoclonal antibodies aimed directly at hepcidin or at the inflammatory stimuli that induce its production, including IL-6 [13].

Table 1 Definitions of anemia in children with kidney disease [22, 23, 65]

KDOQI (2006)		
Age group (years)	**5th percentile Hemoglobin level (g/dL)**	
	Boys	**Girls**
1–2	10.7	10.8
3–5	11.2	11.1
6–8	11.5	11.5
9–11	12.0	11.9
12–14	12.4	11.7
15–19	13.5	11.5
KDIGO (2012)		
Age group (years)	**Hemoglobin level (g/dL)**	
0.5–5	<11.0	
5–12	<11.0	
12–15	<12.0	
>15 and adult	<13.0 (males) <12.0 (females)	
RA (2017)		
Age group (years)	**Hemoglobin level (g/dL)**	
<2	Hb 10.5 g/dL	
≥2	Hb 11 g/dL	

KDOQI, Kidney Disease Outcomes Quality Initiative; KDIGO, Kidney Disease: Improving Global Outcomes; RA, Renal Association

Definition of Anemia in Children with CKD

The application of adult normative hemoglobin thresholds has been shown to substantially underestimate the prevalence of anemia in children with kidney disease, as normal hemoglobin levels vary by age and sex [14]. Table 1 lists hemoglobin thresholds for the diagnosis of anemia per the 2006 National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI), the 2012 Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline for anemia, and the 2017 Renal Association (RA) in the guideline in the United Kingdom.

Evaluation and Treatment of Anemia

Symptoms of Anemia

The clinical presentation of anemia depends on the rate of hemoglobin decline as erythropoietin production slows and the adaptability of the heart and lungs to a decreased hemoglobin concentration. Symptoms of anemia may include fatigue, pallor, somnolence, tachypnea, tachycardia, depression, impaired cognition or muscle function, and loss of appetite. If anemia is more acute from blood loss, patients may experience dyspnea, dizziness, or headache.

Initial Diagnostic Evaluation of Anemia

Prior to initiating treatment with an ESA in anemic children with kidney disease, other potentially correctable causes of anemia should be ruled out, as anemia in children with CKD may not be associated solely with erythropoietin deficiency. A variety of possible causes are listed in Table 2.

Erythropoietin Levels

EPO deficiency is a diagnosis of exclusion. In the setting of normal renal function, plasma EPO

Table 2 Potential causes of anemia in children with chronic kidney disease

Erythropoietin deficiency and/or dysregulation
Iron-restricted erythropoiesis Absolute iron deficiency Functional iron deficiency Impaired iron trafficking
Inflammation and hepcidin upregulation
Chronic blood loss Frequent phlebotomy Hemodialysis circuit losses GI losses Menstrual losses
Acute blood loss Surgical losses GI losses
Uremia and oxidative stress
Hyperparathyroidism and myelofibrosis
Hypervolemia
Nutritional deficiencies B12, folate, carnitine, vitamin C, copper
Medications Angiotensin-converting enzyme inhibitors Nonadherence with anemia therapies Drug toxicity Pure red cell aplasia associated with erythropoiesis stimulating agents
Infectious causes Parvovirus B19-induced aplastic anemia Infection-associated inflammation

levels increase exponentially with decreasing hemoglobin, and values may rise from the normal range of approximately 15 units/liter to as high as 10,000 units/liter [15]. Thus, measuring plasma levels of EPO in children with kidney disease is not useful to clarify the contribution of relative EPO deficiency to anemia, because even if EPO levels are detectable above the normal range, they may still be inappropriately low for the degree of anemia present.

Laboratory Assessment of Iron Status

In clinical practice, the most commonly utilized biomarkers of stored iron remain serum ferritin and transferrin saturation (TSAT). Both ferritin and TSAT have limited sensitivity and specificity to predict bone marrow iron stores and erythropoietic response to iron supplementation. Distinguishing hepcidin-mediated impaired iron trafficking from absolute iron deficiency anemia presents a clinical challenge, as both disorders are characterized by a microcytic anemia with low reticulocyte counts, decreased serum iron concentration, and low transferrin saturation. However, serum ferritin levels can be helpful in distinguishing the disorders; absolute iron deficiency is associated with a low ferritin concentration, while impaired trafficking is characterized by normal or elevated serum ferritin, reflecting iron sequestration in the reticuloendothelial system. In contrast, in patients with functional iron deficiency on ESA therapy, the rate of enteral iron absorption or release from reticuloendothelial cells is inadequate to meet the demands for erythropoiesis; these patients often have low TSAT values with normal or high levels of ferritin, suggesting that patients may benefit from treatment with intravenous iron [16].

TSAT has recognized limitations as well, including diurnal fluctuations and reduction in the setting of malnutrition and chronic disease [17]. There is thus a need for diagnostic tests which more accurately predict the need for or response to iron therapy. A study in pediatric dialysis patients found that the reticulocyte hemoglobin content (Ret-He), which is not an acute phase reactant and reflects iron availability for incorporation into reticulocytes over the previous 2–4 days, performed better than either ferritin or TSAT to distinguish between iron deficiency and sub-optimal ESA dosing [17]. Thus, Ret-He may be an attractive alternative indicator of iron status in clinical practice, although it remains limited currently as it is only measured by flow cytometry [17]. Percentage of hypochromic red blood cells (% HRC) is another laboratory marker of iron status that can assess iron availability for incorporation into red cells. % HRC >6% suggests poor hemoglobin production due to iron deficiency, and may be helpful in distinguishing patients with elevated ferritin levels who may benefit from additional, potentially intravenous, iron supplementation [18]. %HRC also requires flow cytometry for measurement, which may limit its availability in clinical laboratories which measure hemoglobin by electrical impedance and do not have access to the equipment or software required for testing.

Recombinant Human Erythropoietin Therapy

Available Forms of rHuEPO

The human *EPO* gene was isolated in 1985, with commercial production of rHuEPO beginning soon thereafter. Epoetin alfa was approved by the US Food and Drug Administration in 1989 [6]. The development and widespread use of rHuEPO in both adults and children eliminated dependence on red blood cell transfusions which could be complicated by transfusion-associated viral infections, iron overload, and allosensitization [19]. Both endogenous and recombinant EPO have microheterogeneity in carbohydrate structures with variation in sialic acid content, and the molecules with increased sialic acid content demonstrate increased in vivo half-life [20]. The observation that the biologic properties of specific rHuEPO products varied with their molecular structure led to the hypothesis that re-engineering the EPO molecule with the addition of carbohydrate chains, and increasing sialic acid content, could enhance potency and increase half-life. Now there are several types of both short- and long-acting ESA available worldwide, and new formulations continue to emerge requiring ongoing attention to the relative safety and efficacy in children compared to adults.

Epoetin

Epoetin alfa was the first rHuEPO commercialized in the United States, followed by epoetin beta in Europe. Epoetins alfa and beta, both produced in Chinese hamster ovary cells, have minor structural differences but the same physiological effects [20]. There is no definitive evidence of superiority in patient outcomes for any particular epoetin brand. As the patents for the first epoetins expired, biosimilar agents showing only minor differences in clinically inactive components to those of the licensed products emerged and were approved in the United States and Europe; this process will likely continue, leading to wider entry of these agents into the clinical markets [21]. Short-acting epoetin formulations achieve maximum efficacy when dosed 1–3 times weekly and demonstrate a longer half-life when given subcutaneously (19–24 h) than intravenously (6–8 h) [22].

Darbepoetin Alfa

Darbepoetin alfa is an erythropoietin analogue with two additional sialic acid-containing carbohydrates resulting in extended in vivo biological activity. Darbepoetin alfa has been shown to have equivalent efficacy as rHuEPO to maintain hemoglobin when dosed weekly or every other week in children with CKD [23–25]. Darbepoetin alfa may be administered intravenously or subcutaneously, and while the drug clearance, half-life, and bioavailability are similar in adults and children regardless of the route of administration, absorption when given subcutaneously may be more rapid in children [22]. The longer dosing interval inherent to treatment with darbepoetin compared to rHuEPO has made subcutaneous darbepoetin alfa an attractive alternative for anemia management in younger children, with the potential for improving adherence due to the need for less frequent drug administration. A randomized clinical trial in children with CKD stages 4 and 5 demonstrated that darbepoetin alfa is as safe and effective as rHuEPO for the correction of anemia [25]. A longitudinal European registry study similarly demonstrated that children treated with darbepoetin alfa did not experience increased rates of adverse events such as infection or severe hypertension [26]. A potential limitation of darbepoetin alfa in children, however, is the reported discomfort associated with injection. In a blinded, randomized, controlled trial, children who received subcutaneous darbepoetin alfa reported higher levels of post-injection pain by a visual analogue scale compared to those who got epoetin beta, consistent with an increased impression of pain as reported by their parents and nurses [27]. A potential explanation is the more physiological pH of the buffer in the epoetin beta preparation than the darbepoetin alfa preparation [19]. Other pediatric studies have, however, not found pain to be more frequent or severe in patients receiving darbepoetin [25].

Continuous Erythropoietin Receptor Activator

Continuous erythropoietin receptor activator (CERA) is an EPO analog that was created with the integration of a single 30 kDa polymer chain into the EPO molecule, increasing its molecular weight to twice that of epoetin and extending its elimination half-life substantially, to around 130 h [28]. Unlike endogenous EPO and epoetin, which are internalized and degraded following binding to the EPO receptor, CERA escapes degradation by dissociating from the receptor, allowing sustained in vivo efficacy of the compound [29]. A study in children on peritoneal dialysis demonstrated that CERA safely and effectively maintained hemoglobin levels when dosed once or twice monthly, although the doses required to meet goal hemoglobin levels were higher than those required in previously published adult studies [30]. A study in 64 children on hemodialysis aged 6–17 found that CERA given once every 4 weeks was efficacious in maintaining hemoglobin levels with a safety profile consistent with that of adults [31].

ESA Dosing for Children

rHuEPO quantities are traditionally expressed as units, with 1 unit equaling the erythropoietic effect of bone marrow stimulation with 5 μmol of cobalt chloride, an historic treatment for anemia prior to the rHuEPO era [1]. Darbepoetin alfa quantity is expressed in micrograms, with the biological activity of 1 μg corresponding to that of 200 units of rHuEPO [15]. Table 3 lists suggested dosing for ESA agents. The goal after ESA initiation is for a rate of increase in hemoglobin concentration of no more than 1–2 g/dL per month [23].

ESA dose adjustments after treatment initiation should generally not be made until after the first 4 weeks of therapy, and no more often than every 2 weeks in the outpatient setting, as the effects of therapy are not likely to be seen after shorter intervals [23]. Decisions on dosing adjustments should be made based on the hemoglobin rate of rise after initiation, and the stability of hemoglobin during maintenance therapy. When a decrease in hemoglobin is necessary, ESA dose should be decreased but not necessarily held, as a pattern of holding and reinitiating ESA therapy can lead to hemoglobin cycling around the desired target range [32]. Long-acting ESAs like darbepoetin alfa and CERA, with their increased half-life and lower binding affinity for the EPO receptor, stimulate erythropoiesis for longer periods of time and thus may cause higher-than-intended hemoglobin levels in clinical practice. This can be avoided by using lower starting doses and making less frequent dose adjustments than in children treated with short-acting ESAs [33].

Dosing ESA in Children Compared to Adults

The dosing requirements of rHuEPO differ between children and adults. Young children have been reported to require higher weight-related rHuEPO doses than adults, ranging from 275–350 units/kg/week for infants to 200–250 units/kg/week for older children [34]. Despite their lower body weight, children and adolescents on chronic hemodialysis have also been found to

Table 3 Erythropoiesis stimulating agent dosing guide for initiation in children

	Starting Dose	Interval	Route
Epoetin alfa/beta	20–50 IU/kg	3 times per week	SC or IV
Darbepoetin alfa	0.45 μg/kg 0.75 μg/kg	Weekly Every 2 weeks	SC
CERA	4 μg per each prior weekly dose of: 125 IU epoetin 0.55 μg darbepoetin	Every 4 weeks	SC or IV

IU international units; SC subcutaneously; IV intravenously; CERA continuous erythropoietin receptor activator require higher absolute doses of rHuEPO than adults to maintain hemoglobin in goal range [35]. In contrast to typical drug dosing in children which is based on body size to account for a decreased volume of distribution, rHuEPO dose requirements appear to be independent of weight [36]. The potential mechanisms for increased rHuEPO dose requirement in children are not clear, but may include increased presence of non-hematopoietic erythropoietin binding sites (e.g., endothelial, kidney, brain, and skeletal muscle cells) resulting in increased drug clearance, or increased erythropoietin demand during periods of accelerated body growth [15, 34].

HIF Stabilizers

There has been a great deal of interest in the use of HIF stabilizers/prolyl hydroxylase inhibitors (HIF-PHIs) as an ESA-independent treatment for the anemia of CKD, and trials evaluating the efficacy and safety of multiple agents are ongoing. Small-molecule HIF-PHIs modulate HIF-controlled gene products and are capable of inducing endogenous erythropoietin production even in the setting of decreased renal oxygen consumption or even in the absence of kidneys, likely by increasing hepatic erythropoietin production [37]. They can be administered orally in highly bioavailable formulations. Trials in children are in the planning stages, but HIF-PHIs likely represent a promising future treatment avenue in children, particularly in those with ESA-resistant anemia. Longitudinal studies will be necessary to assess for associations between HIF-PHI treatment and risk for potential adverse outcomes including thromboembolic events, oncologic transformation, and pulmonary hypertension.

ESA Hyporesponsiveness

There are patients who, despite iron supplementation combined with escalating ESA dosing, fail to increase hemoglobin levels above 10 g/dL. KDIGO defines *initial* ESA hyporesponsiveness as no increase in hemoglobin concentration from baseline after the first month of appropriate weight-based dosing, and *acquired* ESA hyporesponsiveness as a requirement for 2 increases in ESA doses up to 50% beyond the dose which they had previously required to maintain a stable hemoglobin concentration [23]. An alternative definition is a persistent hemoglobin deficit after 3 months of high-dose ESA treatment (rHuEPO in excess of 400 units/kg weekly or darbepoetin alfa in excess of 1 μg/kg weekly) [38]. While ESA hyporesponsiveness may be chronic, it can also be seen in the context of shorter-term clinical events such as infections or surgical procedures which may negatively impact the response to ESA therapy. Thus, the potential risks and benefits of escalation in ESA dose versus administration of intravenous iron versus red blood cell transfusion in this setting must be assessed for individual patients [23]. Although aluminum-based compounds are used far less frequently in the management of hyperphosphatemia than in the past, awareness of aluminum toxicity is important. Chronic use of aluminum-based antacids and phosphate binders remains the most common cause of aluminum toxicity in patients with ESRD.

Anti-rHuEPO Antibodies

ESA-induced pure red cell aplasia (PRCA) is an increasingly rare hematologic disorder that was first described in the late 1990s. PRCA is characterized by a severe and progressive normocytic anemia, reticulocytopenia, and the almost complete absence of erythroid precursors in the bone marrow, with affected patients becoming transfusion dependent [39]. ESA-induced PRCA is secondary to the development of neutralizing antibodies which block the interaction between an ESA (including epoetin alfa or beta, darbepoetin alfa, or endogenous EPO) and its receptor [39]. Most initial cases of ESA-induced PRCA were seen in countries where epoetin alfa formulated with a polysorbate 80 stabilizer was administered to CKD patients subcutaneously; regulatory advisories have subsequently discouraged this practice [40].

Red Blood Cell Transfusion

Despite best efforts in anemia management, and often in the setting of ESA hyporesponsiveness, patients sometimes do require packed red blood cell transfusion. The decision to transfuse should not be based on an arbitrary hemoglobin level, but rather guided by symptoms and after weighing the specific risks and benefits for the individual patient. Red blood cell transfusions are associated with an increased risk for the development of human leukocyte antigen (HLA) antibodies. In adults, leukoreduction of blood products is an ineffective means to decrease HLA sensitization, and red cell transfusions lead to clinically significant increases in HLA antibody strength and breadth [41]. These antibodies serve as a barrier to future transplantation and may adversely affect graft outcomes [42]. More studies are needed to define the risks associated with red cell transfusion in children with regard to HLA sensitization and graft outcomes.

Iron Supplementation

Targets for Biomarkers of Iron Stores

For all pediatric CKD patients with anemia *not* on iron or ESA therapy, KDIGO recommends oral iron (or IV iron in children on hemodialysis) administration when TSAT is $\leq 20\%$ and ferritin is ≤ 100 ng/ml [23]. For all pediatric CKD patients *on* ESA therapy who are not receiving iron supplementation, KDIGO recommends oral iron (or IV iron in children on hemodialysis) administration to maintain TSAT $>20\%$ and ferritin >100 ng/ml [23].

Oral Iron Supplementation

Most children will require iron supplementation as part of their anemia treatment plan in order to maintain hemoglobin levels and replete iron stores. Children on hemodialysis in particular may have chronic blood loss via the dialysis circuit which exacerbates iron deficiency. Enteral iron supplementation is relatively inexpensive, highly available and generally safe and efficacious in children with chronic kidney disease, although GI side effects of nausea or constipation are sometimes reported. Although true intolerance is relatively rare, it may be a contributing factor to the poor adherence that may arise to prescribed oral supplementation. In addition, co-administration of iron with phosphate binders or antacids can limit absorption due to changes in gastric pH [43]. The usual dosing range for oral iron supplementation is 3–6 mg/kg/day of elemental iron, either daily or divided into two daily doses. The most commonly available oral iron preparations come in ferrous ($Fe2^+$) or ferric ($Fe3^+$) forms, including ferrous sulfate and ferric iron polymaltose complexes [44]. Ferrous sulfate has better bioavailability (10–15%) than ferric iron, and is available in prolonged release forms [44]. Some ferric polymaltose complex formulations are available with added vitamins C, B12, and folic acid to enhance iron absorption and replete other vitamins associated with red blood cell production. However, there is no evidence that ferric iron formulations are superior to ferrous preparations for oral supplementation in children on dialysis.

Intravenous Iron Supplementation

Children on dialysis often benefit from iron preparations administered intravenously due to the poor enteral absorption or poor tolerance associated with oral administration. There are an increasing number of available iron preparations for clinicians opting for intravenous therapy. Early IV iron compounds were formulated as inorganic iron oxyhydroxide complexes, which could result in the release of labile iron directly into the plasma leading to the formation of highly reactive free radicals associated with severe toxicity, including hypotension. Newer preparations surround the iron oxyhydroxide core with carbohydrate shells of different sizes and polysaccharide branch characteristics [45]. The shell characteristics determine how long the iron remains circulating, with larger molecular weight formulations such as iron dextran resulting in longer plasma residence, while products with smaller shells are more labile and likely to release iron directly into the plasma before it can be metabolized in the reticuloendothelial system [46]. Intravenous iron therapy can be delivered as a loading phase, delivered using consecutive doses to replete iron stores, or as smaller maintenance doses given weekly.

Safety of Intravenous Iron

Given trial evidence in adults that higher ESA doses are associated with adverse cardiovascular outcomes, using IV iron to reduce ESA dose requirements is an attractive treatment option. However, the administration of IV iron, even with new formulations, is not without risk. Concerns about potential adverse effects of IV iron supplementation include iron overload, adverse reactions including anaphylaxis, and increased risk when administered in the context of active infections. There have not been any pediatric studies focused on the long-term effects of IV iron supplementation in patients on chronic dialysis. More research to evaluate the effect of differences in formulation and pharmacokinetics of such agents and to determine whether repeated induction of oxidative stress has longer-term sequelae in terms of inflammation and cellular and tissue iron deposition.

Emerging Routes of Iron Supplementation

Ferric pyrophosphate citrate (FPC, Triferic®) was approved by the US FDA in April 2016 for the replacement of iron to maintain hemoglobin in adults on hemodialysis [47]. FPC is dissolved in dialysate and administered via dialysis. In contrast to the IV iron administration of other iron preparations which can lead to the presence of non-transferrin bound iron in the circulation and potentially trigger the generation of pro-oxidant and atherogenic molecules, FPC is quickly transferrin bound and removed from the circulation [48]. Pratt et al. conducted a pediatric study in which FPC was administered via the dialysate (added to bicarbonate concentrate) or intravenously (via the venous blood return line) to 22 hemodialysis patients <18 years of age during dialysis treatment and found it was well tolerated [49]. The mean serum total iron concentrations peaked 3–4 h after administration, and iron exposure based on maximum serum concentration was greater after dialysate administration compared to the intravenous route [49]. Serum iron concentrations were also higher in 12- $<$ 18-year-old subjects after dosing compared to $<$12-year-olds [49].

Ferric citrate is a novel oral phosphate binder which also supplies elemental iron, and has the potential benefit of providing therapy for at least two CKD co-morbidities in a single agent, a potentially adherence-enhancing strategy. The ferric ion in ferric citrate combines with dietary phosphorus in the GI tract, but the excess ferric ions are reduced by the bowel mucosa to ferrous iron and absorbed into the systemic circulation [50]. Ferric citrate has been approved by the US FDA for use in adults, but the safety and efficacy are in the process of being systematically assessed in children with CKD. In a randomized controlled trial conducted in 60 adults with mild-to-moderate CKD (eGFR 15–45 ml/min/1.73 m^2) and iron deficiency (defined as TSAT $\leq$30% and ferritin $\leq$300 ng/ml), 12 weeks of treatment with ferric citrate resulted in more robust increases in TSAT, ferritin, and hepcidin compared to ferrous sulfate treatment [51]. No differences in either mean change in hemoglobin or adverse events were noted between groups, with the most common reported adverse event gastrointestinal complaints [51].

Anemia after Successful Renal Transplantation

Post-transplantation anemia (PTA) is a frequent complication among both adult and pediatric kidney transplant recipients and is usually more common immediately after transplantation in spite of early stabilization of graft function. Depending on time after transplantation *early PTA*, usually defined as anemia which develops up to 6 months after transplantation, can be distinguished from *late PTA*, defined as anemia which develops after 6 months [52]. Causes of and risk factors for PTA are multifactorial and are summarized in Table 4.

Table 4 Risk factors for early and late post-transplantation anemia (PTA)

Early PTA (<6 months)	Late PTA (>6 months)
Pretransplant anemia	Early PTA
Iron deficiency – poor nutrition	Impaired GFR
depletion of iron stores before Tx	Iron deficiency
surgery blood loss	Maintenance immunosuppression (MMF, AZA)
frequent blood samples	Ace-inhibitors
Induction immunosuppression	Acute rejection
Virus and bacterial prophylaxis	Infections
Infections	Comorbidities
Delay graft function	Chronic inflammation
Acute rejection	Hyperparathyroidism
Hyperparathyroidism	
Female gender	
Younger age	

Clinical Outcomes

PTA has been associated with multiple long-term outcomes: all-cause mortality, decline in estimated eGFR, allograft dysfunction and loss, left ventricular hypertrophy, and congestive heart failure [53]. Many retrospective and a few prospective adult studies have investigated and confirmed the association between PTA and mortality and graft failure [54]. Anemia may contribute to hypoxic damage secondary to reduced oxygen delivery to the tubulointerstitium which stimulates further production of proinflammatory and profibrotic molecules, perpetuating chronic allograft injury [55]. Anemia has been identified as an independent risk factor for the development of left ventricular hypertrophy and de novo congestive heart failure, both strong predictors of mortality among kidney transplant recipients. Even modest reductions in hemoglobin are recognized as hemodynamic stressors that promote ventricular growth and remodeling, ultimately causing left ventricular dilation and likely contributing to increased cardiac mortality [56, 57].

Evaluation and Prevention of PTA

The initial diagnostic work-up of PTA is similar to that suggested in pre-transplant CKD (Table 2). Transplanted patients should be screened frequently for anemia. Recommendations include at least weekly for months 1 to 2, at least every other week for months 3 to 4, at least monthly for months 4 to 12, and then at least once every 3 to 6 months [58] in order to start early treatment and improve prognosis [53]. Effective management of pre-transplant anemia may prevent the development of PTA. Implementation of anemia treatment to properly prepare candidates for kidney transplantation protects against the consequences of intraoperative blood loss and hemodilution, which includes the need for post-operative blood transfusions [59].

Treatment of PTA

Considering its multifactorial nature, PTA treatment may be challenging and should be directed to the underlying cause. There are not specific recommendations for anemia treatment in transplanted patients and PTA is usually treated in the same manner as anemia in CKD. Treating mild and severe anemia in patients >3 months following successful transplantation may increase their survival probability and reduce the rate of decline of GFR [60]. There is no contraindication to ESA therapy for the treatment of PTA [61]. Despite a high prevalence of anemia post-transplant, the number of patients receiving ESA therapy in the post-transplant period is low, less than 10% [62] and the optimal timing of initiation and the effectiveness of ESA therapy has not been defined. In this setting, the presence of significant interstitial fibrosis and tubular atrophy on transplant biopsy may be a useful indicator of the need for ESA treatment [63]. Every effort should be made to reduce the need for red cell transfusions in renal transplant recipients. Adverse consequences of perioperative transfusions include the development of donor specific antibodies, with antibody-mediated rejection more frequently detected in transfused patients than in non-transfused recipients [64].

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Assessment of Volume Status in Chronic Kidney Disease

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Fabio Paglialonga and Manish D. Sinha

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Abstract

Overhydration and dehydration remain a difficult balance to maintain for children with chronic kidney disease (CKD). For clinicians managing children with CKD, especially those on dialysis, an accurate assessment of volume continues to be one of the most difficult issues and one that needs regular evaluation to maintain optimal sodium and water homeostasis. Traditional tools including clinical history, physical examination, and measurement of blood pressure may not provide an accurate assessment of fluid overload, which remains one of the main drivers of arterial hypertension

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in children with CKD and those receiving kidney replacement therapy (KRT). Various techniques have been proposed to assess volume accurately in these patients including single- and multifrequency bioimpedance analysis and, for children on hemodialysis (HD), blood volume monitoring. Both have gained much attention over the past few years, while limited experience and scarce data are available for lung ultrasound, inferior vena cava echography and use of N-terminal pro-brain natriuretic peptide in children with severe CKD and those requiring dialysis. For the clinician, it remains important to remember that patients often have a combination of etiologies (e.g., volume overload and increased renin). A detailed clinical history and examination, supported by serial measurements using one or more of the above techniques of volume assessment, can guide management and maintain the child in an euvolemic state, avoiding the deleterious effect of fluid excess or depletion. Currently, no single technique can be considered as gold standard, but each method, at best, contributes to improving volume assessment when repeated over time on the same patient.

Keywords

Peritoneal dialysis (PD) · Hemodialysis (HD) · Child · Intravascular volume · Overhydration · Body composition monitoring · Interdialytic weight gain

Introduction

Childhood chronic kidney disease (CKD) is a devastating illness requiring lifelong medical input, and despite improved survival, those receiving kidney replacement therapy (KRT) continue to display excess morbidity and mortality secondary to cardiovascular (CV) disease [1, 2]. Longitudinal studies report that ~45% of deaths are from CV disease, increasing to 57% in children on hemodialysis (HD) [3, 4]. In children with nondialysis CKD, left ventricular hypertrophy (LVH) is common and occurs early in the disease process with reported prevalence of up to 65% and increasing to 82% in those on HD [5, 6]. Arterial hypertension is observed in >50% children [7, 8], and uncontrolled or difficult to control hypertension is one of the major risk factors for cardiovascular disease in this cohort. Available longitudinal data for children on long-term KRT largely describes a progressive deterioration in cardiac structure and function with increasing time on dialysis, with few data showing improvement [9, 10].

Optimal management of sodium and water homeostasis is a priority in children with CKD and in particular for those on dialysis. Both water and sodium are major components to be considered when performing a comprehensive evaluation of a patient's volume status [11]. Similar to the general population in whom dietary salt reduction has been shown to lower blood pressure (BP) and CV risk, in adults receiving chronic dialysis, salt reduction has also been shown to be associated with systolic and diastolic BP reduction [12]. The assessment of sodium balance is very difficult to obtain in clinical practice, and volume assessment is usually restricted to fluid status evaluation. It is however important to recognize that reduction in sodium intake is essential to the reduction in the patient's interdialytic weight gain (IDWG).

The majority of childhood etiologies causing CKD are "salt losing," and as opposed to adults, more children on KRT remain polyuric. Despite this, most children, once started on dialysis, will experience a reduction in urine output and develop relative oliguria or become anuric [10]. Assessment of the euvolemic status in a child on dialysis relies on the identification of so-called dry weight, that is, the optimal body weight to be aimed for at the end of a dialysis session. For clinicians, this remains one of the most challenging aspects in children with end-stage kidney disease (ESKD) management, with major obstacles faced as a result of the low sensitivity of clinical markers and the lack of a gold standard method or even a clinically reliable method of sufficient accuracy to estimate fluid overload across the childhood age range. In contrast to adults on dialysis, additional peculiarities of the pediatric population include challenges around the clinical evaluation of young children, limitations in their ability to tolerate investigations, changing body composition with growth through childhood, and rapid changes in hemodynamic measures when unwell. This underscores the major clinical relevance of the assessment of fluid status in a dialysis-dependent child.

The goal of sodium and water management in children with CKD is to keep the child in the narrow window between volume overload and volume depletion. Both conditions are associated with significant complications, and both can lead to a reduced quality of life and even increased mortality in CKD. Given the low level of evidence from current studies in children, no clear consensus exists concerning the optimal technique of volume assessment in the pediatric CKD population.

The management of volume overload in children on peritoneal dialysis and HD is discussed under the relevant chapters.

Consequences of Volume Overload

Patients with oligoanuric CKD and those on dialysis develop an increase in extracellular volume as a consequence of the loss of renal regulation of salt and water. The resultant cardiovascular complications include not only hypertension, LVH, and large arterial disease [1, 13] but also cardiac failure and pulmonary edema in cases of severe volume overload. In children treated with dialysis, both post-dialysis fluid overload and IDWG remain significantly correlated with BP and markers of cardiac and vascular health, although the data remain conflicting likely due to small patient numbers [14, 15]. Other possible consequences of volume excess include increased inflammation, loss of appetite, and malnutrition. This risks progression is a vicious cycle linking volume overload, reduced appetite, and resultant loss of "real" body weight. As these changes can be difficult to assess clinically, this may result in further worsening of volume excess and thus should always be kept in mind in patients on dialysis, and dry weight should be adjusted regularly. Furthermore, if a high ultrafiltration (UF) rate is prescribed in case of severe fluid overload, it carries a risk of hypovolemia and its consequences that are described below.

Consequences of Excessive Volume Depletion

Hypovolemia is common in children with salt-wasting nephropathies, those treated with diuretics, and those in whom UF is performed during dialysis. Hypovolemia may be associated with several acute symptoms, including asthenia, fatigue, dizziness, cramps, nausea, and vomiting. Intradialytic hypotension can cause hypoperfusion of several organs, including the brain, eyes, gut, kidneys, and the myocardium. The loss of residual kidney function is a particularly dramatic complication given the superiority of kidney clearance over dialytic clearance on uremic toxins and the importance of residual kidney function in volume management itself. Myocardial stunning, a segmental decrease of myocardial contractility, occurs in the majority of HD sessions even in children [16]; it is more common when high ultrafiltration rates are used, and in the long term, it may be associated with severe cardiac impairment, as myocardial hibernation and fibrosis. Brain hypoperfusion has been associated with watershed infarctions in patients on HD undergoing intradialytic hypotension [17]. Anterior ischemic optic neuropathy is a rare but dramatic complication for patients undergoing PD, characterized by infarction of the optic nerve head due to hypoperfusion of the posterior ciliary arteries and causing sudden blindness, usually driven by hypotension [18].

Access malfunction and thrombosis are other possible complications of hypovolemia in children treated with HD, which deserves attention given the importance of preserving vascular access in children with a foreseeable long period of KRT ahead.

Clinical Assessment of Volume Status

History and Physical Examination

Clinical assessment remains of utmost importance in children with CKD and those receiving dialysis. For those on dialysis, a "dry weight" is defined as the lowest weight a patient can tolerate without the development of symptoms or hypotension. It is, at best, an estimate and needs constant evaluation and regular adjustment, particularly in the growing child. Readjustment of a child's dry weight is usually based on a combination of clinical assessment, evaluation of events during dialysis, and investigations to assess volume using some of the methods described later. Physical examination should include evaluation for the presence of edema, degree of filling of the jugular vein, and lung auscultation. It is important to remember that edema is often not clinically evident until the extracellular fluid overload has exceeded 10% of body weight [19]. Although important, the clinical assessment may be grossly misleading, particularly in less experienced hands, and should not be considered sensitive enough on its own to provide an adequate assessment of volume in children with CKD.

It is essential to regularly review the child's 24-h urine output. This should be clearly documented at the time of commencement of dialysis and ideally repeated at 3-monthly intervals as it is likely to reduce over time. Based on urine output, patients can be categorized as either anephric, oliguric (<300 ml/m^2/day), normal, or polyuric (over 2 l/m^2/day). Despite its clinical utility, 24-h urine output is often difficult to collect or not possible especially in the youngest patients. Insensible fluid losses calculated at 400 ml/m^2 body surface area should be taken into account to obtain an accurate fluid balance. Fluid allowance should be clearly documented and regularly discussed with the child and their caregivers. Weight gain between HD sessions, called IDWG, is ascribed to be secondary to fluid gain between dialysis sessions [20] and is a simple measure of intermittent volume gain in patients receiving intermittent maintenance HD. In small studies in children receiving HD, IDWG has been shown to correlate with BP and left ventricular mass index, and those with a median IDWG $>4\%$ of body weight are at higher risk of LVH [14].

The occurrence of any event potentially leading to weight loss (e.g., intercurrent infections, diarrhea, and vomiting) or weight gain (e.g., acute kidney injury resulting in reduction in urine output) should be investigated comprehensively. In the same way, any symptoms of volume excess and depletion should be looked for, as previously described. An accurate fluid balance, taking into account fluid intake and changes in 24-h urine output, should be performed. An assessment of appetite, including an evaluation of dietary intake of energy and protein and the change in intake, may be of help in interpreting or predicting changes in body weight. When unwell, changes in *true* body weight can be particularly rapid in young children receiving dialysis and especially in those who may have restricted diets or those dependent on protein and calorie supplementation for their nutrition. The clinician will often need to "reset" the target "dry weight" to avoid the inevitable water logging and consequent hypertension.

Blood Pressure

An accurate assessment of BP is critical, but some issues should be considered further. Firstly, 24-h ambulatory BP measurement (ABPM) remains the gold standard method for accurate assessment of hypertension in this population with a high prevalence of masked hypertension [10]; interdialytic 44-h ABPM may provide even more information than 24-h ABPM in diagnosing hypertension in pediatric patients on maintenance HD [21], although tolerance of these and repeat measurements in the pediatric population are less likely to be successful and therefore not widely used in clinical practice but should be considered in individuals with difficult or resistant hypertension. While desirable, ABPM cannot be performed in the youngest patients on dialysis and those with limiting comorbidities. Home BP may be a possible alternative, with higher agreement with ABPM compared to office BP [22]. In particular, intra- or post-dialytic BP should be viewed as markers of dialytic stability only, as they do not accurately reflect the true BP level of the patient.

Next is the complex relationship between volume and BP. Hypervolemia as a result of excess fluid intake or inadequate removal of salt and water during dialysis remains one of the most important mechanism underlying increased BP levels. Although a number of mechanisms have been implicated in adult dialysis patients, including activation of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system, endothelial dysfunction resulting in abnormalities of nitric oxide production, and vascular stiffening, hypervolemia in children is considered the primary mechanism by most experts [23]. Even in patients with volume overload, the lag phenomenon should be considered: this is the delay in BP reduction after normalization of volume status, due to vasoconstriction. The opposite phenomenon has also been observed, in that fluid loading does not necessarily acutely raise BP. Some patients have normal BP despite hypervolemia, although in the published literature the assessment of this hypervolemia has not been systematically demonstrated and it is unclear if this equates to an "increased intravascular volume." In this scenario, the BP may be viewed as an important index, but basing management decisions on BP values alone may be misleading: the presence of hypertension does not necessarily denote euvolemia, and normotension does not necessarily exclude volume overload. A particular case is the so-called phenomenon of drug-resistant hypertension in children with ESKD, which should be considered volume-dependent until proven otherwise. Finally, other causes of relevance in the child receiving dialysis should be considered. For example, in those with an underlying glomerular disease, hypertension may be disproportionate to the degree of volume loading and secondary to excess renin production. Importantly, a patient with impaired left ventricular systolic function may not be hypertensive despite significant fluid overload.

Techniques to Assess Fluid Status

Given the limitations of clinical parameters in the assessment of volume status, it is essential to rely on technology to improve fluid evaluation. Among the various proposed techniques, bioimpedance analysis (BIA), blood volume monitoring (BVM), lung ultrasound (LUS), inferior vena cava (IVC) echography, and N-terminal pro-brain natriuretic peptide (NT-proBNP) are currently widely used in pediatric nephrology centers. Some of these methods aim at measuring intravascular volume (BVM, IVC diameter, BNP), while other techniques assess total body water or extracellular water. None of these methods can be considered gold standard techniques: the main advantages and limitations of each technique are summarized in Table 1.

Bioimpedance Analysis (BIA)

BIA is a noninvasive bedside technique for the assessment of body composition. Two main approaches have been proposed: single-frequency and multifrequency BIA. Single-frequency BIA uses an impedance plethysmograph that emits

Table 1 Comparison of techniques for assessment of fluid status in children with CKD

Techniques for assessment and body "compartments" assessed	BIA ECW/TBW	LUS ECW-TBW	BVM Intravascular space	IVC US Intravascular space	NT-proBNP Intravascular space
Reproducibility	+++	++	+++	+	+++
Ease of application	++	++	+++	+	+++
Time needed	++	+	+++	++	+++
Ease of interpretation	++	++	+	+	+
Applicability to CKD and PD	++	++	+	++	++
Sensitivity to volume status	++	++	++	+	+
Evidence in children	++	++	+++	+	+

+: poor, ++: moderate, +++: good. *CKD* chronic kidney disease, *BIA* bioimpedance analysis, *LUS* lung ultrasound, *BVM* blood volume monitoring, *IVC US* inferior vena cava ultrasound, *NT-proBNP* N-terminal pro-brain natriuretic peptide, *ECW/TBW* extracellular water to total body water ratio, *PD* peritoneal dialysis

800 mcA and 50 kHz alternating sinusoidal currents. With the subject in a supine position, the outer electrodes are placed on the proximal, dorsal surface of the third proximal phalanx of the right hand and foot and the inner electrodes on the wrist and ankle in such a way as to leave at least 5 cm of free skin around the outer electrodes. Impedance is measured and its components resistance (R) and reactance (Xc) given as output measures. Resistance (which represents the opposition to flow of an alternating current through intra- and extracellular ionic solutions) is relatively low in lean tissues, which contain large amounts of water and electrolytes, and relatively high in adipose tissue and the bone. Reactance, the opposition to flow of an electric current due to capacitance, is produced due to the insulating effect of cell membranes, which act as capacitors. Thus, both indices increase as body water decreases and vice versa. Values of R and Xc can be used as absolute values or placed in a vector plot. Although some reports show that single-frequency BIA could help in improving dry weight assessment in children on dialysis [24, 25], this technique is not widely used, mainly due to difficulties in the interpretation of the data. Multifrequency bioimpedance, and in particular BIA spectroscopy (BIS), consists in the measurement of impedance through a wide range of frequencies, thus allowing for the differentiation between extracellular and intracellular compartments and, by means of predictive equations, the estimation of body compartments, in particular extracellular water, intracellular water, and relative overhydration.

Given its simplicity, BIS has gained popularity in recent years. Although some trials, even in children, have shown a significant correlation between BIA estimates and other markers of hydration [26], a constant finding in the literature is a good agreement at a mean population level but poor individual-level agreement between reference methods and BIS [27, 28]. An attractive clinical utility for BIS would be to evaluate its agreement with IDWG, but these are poorly correlated and thus not currently useful in the estimation of the degree of overhydration. Huge discrepancies have also been found in some cases between BIA estimates and reference techniques, like deuterium bromide dilution technique, so great caution is always needed when using BIA results to determine a patient's dry weight [27]. In general, BIS studies show better accuracy in healthy children than in patients on dialysis. Further studies are needed to better understand the role of BIA in children with CKD. As a general rule, the longitudinal analysis of BIA data in the same patient seems the best strategy for its use.

Blood Volume Monitoring (HD Only)

This technique describes the continuous monitoring of blood volume (BV) changes during an HD session and is based on the measured changes of hematocrit, hemoglobin, or total protein levels of the patient during UF. Relative BV reduction during HD depends on UF rate and refill capacity, the latter depending mainly on plasma oncotic pressure and hydrostatic pressure, which is proportional to tissue hydration: in the situation where the UF rate exceeds refill capacity, hemoconcentration occurs and BV decreases. Most modern HD machines have integrated blood volume monitors that provide a continuous curve representing the percent blood volume change during the session, although the ability to perform BVM may be limited when using the smallest caliber lines and in some older HD machines.

One of the main problems with BVM is that, although some pediatric reports showed that UF with a BV change <8%/h during the first hour of dialysis and <4% thereafter is usually safe, the high inter-patient variability makes it impossible to define a specific threshold, which fits all patients. The shape of the BV curve is probably more informative. In the majority of asymptomatic HD sessions, a common pattern is seen: an initial drop in BV, followed by a plateau, which reflects a perfect balance between UF and refilling. A flat curve, without any reduction of BV, is usually suggestive of volume overload, while a continuous decrease of BV without stabilization suggests very rapid UF and is often associated with intra-HD morbidity (Fig. 1). Both increasing tachycardia and a steeper BV fall in

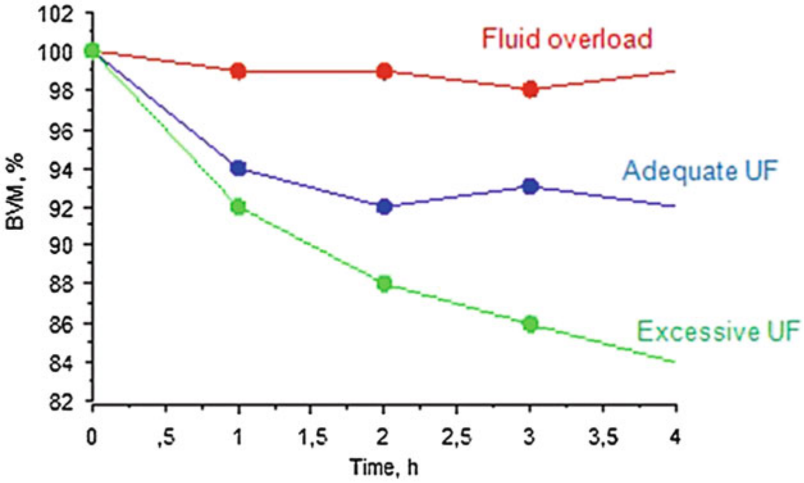

Fig. 1 Typical blood volume monitoring curve shape in case of appropriate ultrafiltration, volume overload, and excessive fluid removal during hemodialysis

the first hour of dialysis have been reported to be associated with hypotension, with or without symptoms [29].

Many studies have demonstrated that the use of BVM in children on HD is associated with better BP control and a lower left ventricular mass index, suggestive of better volume control, and with reduced incidence of intradialytic symptoms [29–31]. This technique can therefore be considered an essential tool to guide UF in children treated with HD.

One of the major advantages of this technique is to have the ability to monitor intravascular fluid volume during an HD session and therefore to provide a more patient-centered therapy. Currently, none of the other techniques allow this to be performed "intra-dialysis" in a practically feasible way but are limited to a pre- and post-dialysis session assessment.

Inferior Vena Cava Echography

Ultrasound assessment of IVC parameters was proposed as a tool to estimate intravascular volume. The end-expiration (maximal) and end-inspiration (minimal) IVC diameters can be measured and the IVC collapsibility index calculated as (maximal diameter − minimal diameter)/maximal diameter. The measurements can be normalized to body surface area and compared with the proposed reference values for healthy children. A decrease of IVC diameter and a reduction of collapsibility index have been shown in children treated with PD or HD after fluid removal, but the reports investigating the role of this technique in fluid assessment are conflicting [32–34]. The main limitation of IVC ultrasound is its low reproducibility and the need to be performed by highly skilled personnel. A second limitation is that it looks at the intravascular compartment only, which is poorly correlated with total body water. Third, the high inter-patient variability makes it difficult to interpret a single IVC diameter value but requires serial measurements.

Lung Ultrasound

The rationale for adopting ultrasound to measure lung water is that water accumulation in the lung interstitium thickens the interlobular septa. This thickening produces a reverberation of the ultrasound beam and generates visible bundles at narrow basis going from the probe to the edge of the echotomography screen: these artifacts are known as "B-lines," and their number is correlated with the amount of extracellular lung water in adults. B-lines can be seen on LUS before lung congestion becomes clinically detectable by auscultation or chest X-rays. B-lines were usually quantified in 14 intercostal positions on the anterior and lateral chest wall for patients weighing under 20 kg and 28 positions for patients over 20 kg.

Recent trials in adult patients with ESKD demonstrated a strong correlation between the number of B-lines and various echocardiography parameters including left atrial volume, pulmonary artery pressure, and ejection fraction and, even more importantly, with both the risk of mortality and cardiovascular complications [35, 36]. The experience in children is so far limited to a couple of studies involving children treated with dialysis, which found a good sensitivity of LUS in detecting subclinical fluid overload [37, 38]. Some important advantages of this technique are its ease of learning and the acceptable inter-observer variability. Unfortunately, LUS is unable to discriminate euvolemia from underhydration. Further studies are needed to better understand how to use this technique to assess dry weight in children.

N-Terminal Pro-brain Natriuretic Peptide (NT-proBNP)

NT-proBNP is synthesized and released from the ventricular myocytes in response to increased ventricular wall stress. Increased left ventricular volume or pressure overload, such as occurs in congestive heart failure and hypertension, enhances the secretion of NT-proBNP from the ventricle. NT-proBNP is a strong predictor of heart failure and mortality in adults with ESKD. Its association with fluid status in adults is still under debate, but according to the available evidence, it should be considered a poor volume marker in this population [39, 40]. The role of NT-proBNP in children on dialysis has been poorly investigated, with conflicting results [25, 41]. At present, there is no published evidence to support its use as a volume marker in children with CKD, and further data are needed.

Conclusions

Fluid status assessment is an essential step in the management of children with ESKD, particularly in those on dialysis. History and physical examination, together with BP assessment, are of utmost importance but not sensitive enough to capture early changes in volume status. Several techniques have been proposed, but this remains largely an underexplored area, and to date, no technique is accurate enough to be considered gold standard. A comprehensive evaluation, including a combination of methods described above, is recommended. For the clinician, it remains important to remember that patients often have a combination of etiologies (e.g., volume overload and increased renin) and that it is important to have a detailed understanding of the temporal events leading to the clinical issue at hand to help keep the child euvolemic and to avoid the deleterious effect of fluid excess or depletion.

Cross-References

- ▶ Chronic Hemodialysis in Children
- ▶ Evaluation and Management of Hypertension in Children
- ▶ Infant Dialysis
- ▶ Peritoneal Dialysis

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Peritoneal Dialysis

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Abstract

Peritoneal dialysis (PD) is currently the most commonly used kidney replacement therapy (KRT) in children worldwide. In this chapter, the fundamentals of peritoneal water and solute transport are detailed, including a description of peritoneal membrane function and transport characteristics. The types of dialysis fluid and their impact on membrane preservation over time on chronic PD and the basic concepts that impact dialysis modality choice are presented. Factors influencing timing of dialysis initiation and choice of catheter and other dialysis equipment, as well as remote patient monitoring options, are reviewed. Once PD is initiated, the dose of dialysis delivered should be measured, typically by measuring urea clearance relative to solute distribution volume (Kt/V). The prescription should be tailored based on the patient's peritoneal membrane characteristics as measured through the peritoneal equilibrium test (PET) and considering the intraperitoneal pressure. Determination of the adequacy of the dialysis procedure should also consider nutritional status, growth, and the general well-being of the patient, in addition to the correction of the metabolic derangements of chronic kidney failure. The PD procedure must be carefully monitored to avoid common and potentially life-threatening complications. Catheter exit site infection and peritonitis are among the most important complications that require ongoing monitoring, and this chapter reviews guidelines for preventing and treating these complications. Common catheter and dialysis procedure-related complications are discussed; patient outcome on chronic PD and promising perspectives are presented.

Keywords

Peritoneal membrane · Peritoneal dialysis fluid · Peritoneal equilibrium test · PET · Adapted APD · Peritonitis · Exit site infection · Leakage · Hernia · Encapsulating peritoneal sclerosis · Remote patient monitoring · Outcomes

Introduction

Peritoneal dialysis (PD) is the predominant initial dialytic modality for children with kidney failure [80] based on several advantages. It can be utilized in any age group including neonates and young infants. Catheter placement is simple as compared to the vascular access required in hemodialysis and is not associated with needling-related fear and pain. Cardiocirculatory stability is superior without an extracorporeal blood circuit and lower ultrafiltration rates. PD provides a greater degree of freedom, hospital visits are infrequent, and compatibility with social life and school attendance is good. Except for infants and young children, routine follow-up in the dialysis centers is usually required once per month. On the other hand, home PD requires significant medical and technical knowledge and encumbers families with major responsibility. The PD procedure appears straightforward but requires in-depth knowledge of the technique, treatment targets, and complications; close interaction between families and experienced members of the dialysis center is essential. This chapter reviews current knowledge of the principles of PD, timing and equipment for implementation of dialysis, and determinants of adequacy, biocompatibility, complications, and outcomes of the PD treatment. It moreover provides an overview on recent technological and therapeutic advancements.

Principles of Peritoneal Solute and Water Transport

The function of PD is determined by the peritoneal membrane characteristics and the peritoneal dialysis fluid. The peritoneum lines, the inner surface of the abdominal wall, cover the intraperitoneal organs, the visceral mesentery, and the omentum. The peritoneal membrane surface area is roughly equal to the body surface area. According to computed tomography studies, only 30%–60% of the peritoneal membrane is in contact with dialysate [19] depending on the fill volume. The primary surface in contact with PD fluid is the mesothelial cell monolayer, which has numerous local homeostatic functions and secretes surface-active substances and lubricants such as CA125 to prevent frictions. Microvilli increase the functional peritoneal surface area.

The submesothelium is 150–450 μm thick and contains blood capillaries, lymphatics, and nerves, which in the healthy peritoneum are mostly located within three distinct layers [96]. The primary barrier that solutes and water have to cross is the capillary endothelial cells, which are linked to each other by junctions. Solute transport occurs via the paracellular and the transcellular route. About one-third of the submesothelial vessels are lymphatics, vessels which reabsorb fluid and (macro-)molecules from the interstitium to the systemic circulation. The submesothelial space consists of extracellular matrix, i.e., collagen bundles, hyaluronan and mucopolysaccharides, few fibroblasts, and isolated inflammatory cells. Its role in transport is minor unless significant submesothelial fibrosis has developed with chronic PD, which reduces the osmotic conductance of glucose. The role of the mesothelial barrier in solute and water transport has not yet been established but may be low [29, 45, 61].

Peritoneal capillary blood flow has been reported to vary between 100 and 150 ml/min in adults and is relatively higher compared to other tissues. PD solute transport appears to be preserved even in moderately hypotensive patients [91]. Transport characteristics as described in the peritoneal equilibration test (see section "The Peritoneal Equilibrium Test") primarily depend on the microvessel density of the peritoneum [95]. Newborns and infants have a higher peritoneal vessel density, which explains the higher solute transport and the need of higher dialysate glucose concentrations to achieve adequate ultrafiltration in this age group. Permeability increases during acute peritonitis, possibly due to increased vessel density, and progressively declines with increasing peritoneal fibrosis. Increased lymph vessel density increases solute and water reabsorption.

Peritoneal dialysis fluids contain high concentrations of glucose as the osmotic agent to remove water together with solutes, a buffer for correction of metabolic acidosis and electrolytes to maintain electrolyte homeostasis. Novel PD fluids may contain bicarbonate buffer and icodextrin or amino acid as the osmotic agent. The driving forces of solute and water transport between the dialysis fluid and the capillary are diffusion, osmosis, and convection. Diffusion along a concentration gradient and across a semipermeable membrane such as the peritoneum is called osmosis. The concentration gradient between blood and dialysate is maintained by exchanging the dialysate. It also depends on the residual dialysate volume, i.e., the dialysate remaining intraperitoneally after drainage. Average residual dialysate volume is about 8% of a dwell volume of 1000 ml/m^2 BSA [101], but this may vary considerably from dwell to dwell and between patients. It increases considerably in patients with intra-abdominal adhesions. In such cases, solutes retained within the residual fluid diminish the concentration gradient. This may be especially relevant with cyclers reducing the fill volume with insufficient drainage of the preceding dwell.

Diffusion is a size-selective process; small molecules such as urea and creatinine diffuse faster than larger molecules such as vitamin B12, the so-called middle molecules. Phosphate transport is slow due to a surrounding aqueous layer increasing the effective molecular weight. Solute transport depends on size, chemical and electrical properties, and the abundance and function of intercellular junctions and transmembrane transporters, which collectively define the permeability of the membrane.

The peritoneal transport characteristics can well be described based on a three-pore model [90]. The ultrasmall pores correspond to the water-selective aquaporin-1 (1%–2% of all pores, radius 2–4 Å), while the molecular counterparts of the abundant small pores (90%–95%, 40–60 Å) and large pores (5%, radius 200–300 Å) have not been defined yet. Forty to fifty percent of water transport, and thus ultrafiltration, occurs via the aquaporins, 50%–60% via the small pores, while water transfer via the large pores is negligible. Fluid transport is determined by the crystalloid pressure exerted by the glucose. In the early phase of a dwell, the high concentrations of glucose removes water primarily via water-selective aquaporine-1, which results in a transient decline in solute concentrations of the dialysate, so-called sodium sieving [61]. During the later phase of the dwell, small pore function predominates, and solute concentration increases again in the dialysate. Icodextrin-based solutions contain starch-derived polymers, which create a colloid osmotic pressure removing water and dissolved solutes via the small pores. This is a slow process, but since icodextrin resorption rate is low, osmosis is maintained for 8–12 h. Sodium sieving does not occur.

Small hydrophilic solute transport occurs almost exclusively by concentration gradient-driven diffusion across the small pores and by convection, i.e., the solutes dragged together with the ultrafiltration; therefore, convective purification depends on the amount of UF achieved. The movement of proteins and other macromolecules occurs across the large pores and is driven by hydrostatic forces.

Solute removal further depends on the effective peritoneal surface area, i.e. the total membrane pore area available for peritoneal transport. It increases with dialysate volumes between 800 and 1400 ml/m^2 BSA and is one-third higher in the supine than in the upright position [38], but is not different with neutral pH, double-chamber PD fluids as compared to acidic, single-chamber fluid [102].

Ultrafiltration consists of the bulk movement of water together with permeable solutes dragged along across the peritoneal membrane (convective mass transfer). The primary driving force is the osmotic pressure of dialysis fluid exerted by glucose or icodextrin. The effect of the hydrostatic pressure gradient is usually small unless the intraperitoneal pressure is high (see section “Standardization of Dwell Volume”). Other factors that affect ultrafiltration are the functional membrane surface area recruited and its hydraulic permeability and reabsorption of fluid into the surrounding tissue via lymphatic vessels. In adults on PD, lymphatic fluid absorption is low [115] but increases substantially with declining ultrafiltration capacity [106].

Choice of PD

The International Society for Peritoneal Dialysis (ISPD) practice recommendations suggest that dialysis modality selection in children should be influenced by child/parent/caregiver choice, the age and size of the child, the presence of comorbidities, modality contraindications, expertise of the dialysis team, and the support available for the child and caregiver [15].

Although there are no prospective studies directly comparing outcomes among children receiving maintenance hemodialysis (HD) and PD, PD is the most common dialysis modality used for children worldwide, particularly in emerging countries where access to the dialysis equipment and staff required to perform HD may be limited [13, 15, 23, 56]. PD is particularly advantageous in infants and small children as it avoids the need for a vascular access, which may be difficult to achieve and/or maintain in this age group. PD also avoids the need for exposure to the anticoagulation often required during HD. Studies suggest that children on PD may lose residual kidney function (RKF) more slowly than those maintained on HD [36, 73]. Compared to in-center HD, the timing of the dialysis procedure is more flexible with PD, which may promote participation in family, social, and school activities.

ISPD recommendations appropriately emphasize that the burden of care for children on home dialysis therapies, including PD, must be considered during modality selection [15]. Responsibilities for the caregiver includes care of the PD catheter, performing the dialysis procedure, medical decision-making (e.g., choosing concentration of glucose, screening for infection), and assessing and addressing any technical issues or alarms if an automated cycler is used. ISPD guidelines suggest that formal assessment of the child's and caregiver's perception of quality of life may inform this process [48, 128].

Contraindications to PD include the presence of an abdominal wall or peritoneal membrane defect, e.g., omphalocele, gastroschisis, unrepaired bladder exstrophy, and diaphragmatic hernia. Extensive peritoneal membrane scarring from previous intra-abdominal surgery, with resultant peritoneal membrane failure, also excludes PD as a dialysis modality. Relative contraindications include the presence of ileostomies and colostomies, as an analysis of the International Peritoneal Dialysis Network (IPDN) revealed that among children on PD enrolled in that registry, the presence of a colostomy was associated with an increased risk for peritonitis [20]. Adequate PD may be difficult to achieve in infants and small children with significant intra-abdominal organomegaly [15].

Initiation of Peritoneal Dialysis and Dialysis Equipment

The right time to begin kidney replacement therapy (KRT) and the technical issues are key factors ensuring a safe journey to kidney transplantation, with normal growth and optimal cognitive development.

Initiation of Dialysis

There is no single criterion for deciding the right time to start dialysis for children with CKD. The most commonly used parameter has been an estimated glomerular filtration rate (eGFR) of less than 10–15 ml/min/1.73 m^2 [59]. A recent analysis of data from the US Renal Data Systems (USRDS) demonstrated an increasing percentage of children initiating dialysis at eGFR higher than 10 ml/min/1.73 m^2. Studies from USRDS and the European Society for Pediatric Nephrology/European Renal Association-European Dialysis and Transplant Association Registry show no clinical benefit in children initiation of PD at higher eGFR, but these studies cannot completely control for clinical indications for dialysis initiation that might also influence outcomes and the imprecision of the estimating equations utilized to calculate GFR. The recently published ISPD guidance suggests initiating dialysis in children at an eGFR of less than 10 ml/min/1.73 m^2 or in the presence or uremic symptoms that are refractory to medical care [15]. In children, biochemical variables like hyperparathyroidism, acid-base status, electrolyte imbalance, anemia, and clinical variables like growth, hypertension, cardiovascular status, residual kidney function (RKF), cognitive development, and school performance should all be carefully considered [83]. However, the high tolerance in children to those imbalances could be misleading, and the potential negative impact of the uremic milieu on growth and cognitive development reinforces the concept of using eGFR as a guide to dialysis initiation. In addition, recent studies suggest that the rate of RKF decline, a key parameter of outcome, may be reduced following initiation of PD, compared to the pre-dialysis period [35, 50].

A detailed assessment of the learning ability and educational level of the parent or caregiver is an important point to evaluate before starting PD therapy. The dialysis team should individualize a training plan for the proper training of adults who will perform the procedure at home. The risk of potentially preventable complications particularly exit site infections and peritonitis has been well documented when an adequate training program has not been implemented. The ISPD guidelines 2012 update [127] emphasizes important points related to caregiver training, including that PD training be conducted by a PD nurse with pediatric training experience. A formal teaching program with clear objectives and assessment criteria should be developed, and retraining sessions should be conducted on a regular basis and in particular following an episode of peritonitis.

Catheter Placement

Once the decision to initiate PD has been made, it is recommended that the catheter be placed by an experienced surgeon or by an experienced nephrologist if no such professional exists. Different types of pediatric PD catheters have been developed since the original catheter introduced by Tenckhoff [112]. Catheter configurations typically include either a straight or swan-neck tunnel configurations and either a pigtail-curled or straight intra-abdominal portions. The extraperitoneal segment has one or two Dacron cuffs, although for children double-cuff catheters predominate, with a proximal cuff fixed in the preperitoneal area and the distal one in the subcutaneous tissue. Catheters come in 31 cm length for newborns, 38.9 for infants, 42 cm for school-age children, and 62.5 cm for adolescents and young adults. The length of the catheter for each patient can be estimated by the distance between the umbilicus and the symphysis pubis. A double-cuff catheter with a downward or lateral subcutaneous tunnel is recommended for children on maintenance PD by the 2012 ISPD guidelines [127]. Some authors recommend that the catheter be inserted laparoscopically, with the curled portion positioned deep in the pelvis. Omentectomy is critical to prevent obstruction of the side holes of the catheter. Adult surgeons perform an omentopexy, a procedure not recommended in pediatrics to avoid the complication of a potential midgut volvulus. In infants, the exit site should be located above the diaper area. Catheter function and the absence of leaks are often confirmed after the placement with 1–3 passes of 10–20 cc/kg of dialysate by dialysis staff, who ideally should be present at the operating room to flush the PD catheter [124]. A transfer line and a plastic or titanium connector are also part of the connection system to the dialysis system. Administration of a first-generation cephalosporin such as cefazolin just prior to the surgical incision, as described in the ISPD guidelines, is recommended [127]. Vancomycin is an alternative in *Staphylococcus aureus* carriers, as well as in hypersensitivity or resistance to cephalosporins.

Starting PD, Cyclers, and Equipment

Unless there is an urgent need to start dialysis, leakage is a serious complication in PD, especially for infants, and delaying the start of therapy is strongly recommended. If dialysis must be initiated urgently, the recommended initial exchange volumes are 10 ml/kg the first day(s), followed by a slow increase of fill volume depending on the individual clearance needs and risks of leakage (see section “Standardization of Dwell Volume”).

An overview on different modes of PD is given in Fig. 1. Continuous ambulatory peritoneal dialysis (CAPD) is performed using a Y-set of tubing. The Y-set is a simple Y-shaped tube with three branches, with one of them being connected to the catheter through a titanium Luer Lock adaptor. Of the other two branches, one is connected to the fresh dialysate bag and the other to a drainage bag or an open container that collects the drained dialysate. Once the dialysate is drained, “fresh” dialysate is used to flush the tubing before fluid is instilled in the abdomen, the so-called “flush before fill” technique [127]. Automated PD (APD; see Chap. 5) is done overnight using a machine (cycler) that fills the peritoneal cavity with dialysis solution, and after a selected dwell time, the solution is drained, and then, the peritoneal cavity is filled with fresh dialysis solution (nightly intermittent PD, NIPD). When a long daytime dwell is added after the short nocturnal APD dwells, it corresponds to a continuous cycling peritoneal dialysis (CCPD) modality. Cycles performed over a residual volume correspond to tidal PD, a modality which may reduce drain alarms in case of catheter dysfunction. The rationale and mode of adapted APD are described in Chap. 5.7. The dwell time and volume and the number of dwells are prescribed according to the transporter status, intraperitoneal pressure, and mathematical calculations described in Chap. 5.

Various automated PD machines are available in the global market; however, they are all based on the same principle. They allow for infusion volumes ranging from 25 to 3500 ml, depending on the cycler, and are equipped with automated spiking or connecting bags, recognition of barcoded bags, and in-line heating of dialysate.

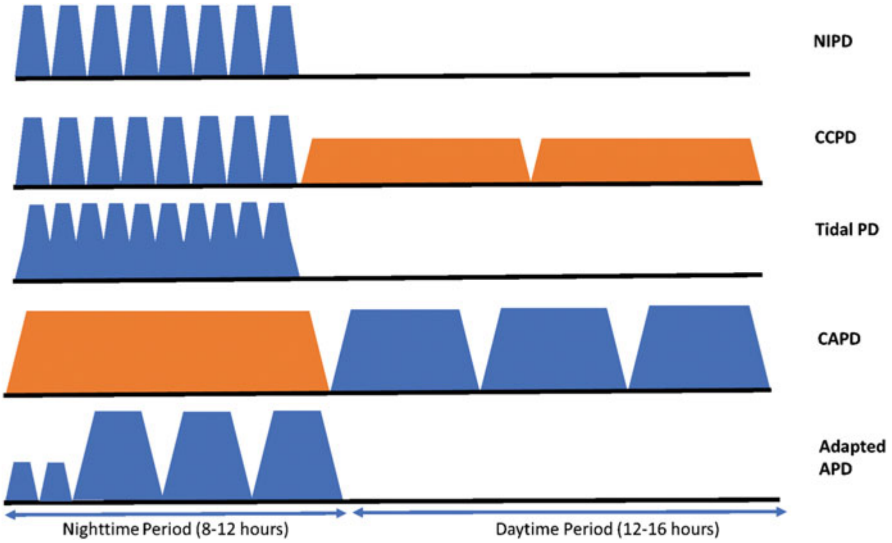

Fig. 1 Schematic illustration of the different modes of PD, based on individual patient's need. To achieve ultrafiltration with tidal PD, intermittent complete drainage may be performed

The most advanced machines have been equipped with remote online monitoring of the procedure (see Chap. 7.2).

Choice of PD Solutions

PD fluids allow for sufficient removal of water and toxins across the peritoneal membrane but due to their highly unphysiological composition (see Tables 1 and 2) result in progressive peritoneal tissue alterations, limiting PD long-term efficiency and sustainability (see Chap. 8). The European Pediatric Dialysis Working Group recommendations therefore emphasize the need for careful PD fluid selection, applying the lowest glucose concentrations and the lowest number of cycles required for adequate ultrafiltration and resulting in the lowest possible cumulative glucose load [100]. In adults, icodextrin solutions significantly increase ultrafiltration and reduce fluid overload. Icodextrin fluids are also well tolerated in children and may therefore be used once daily during the long dwell. To further reduce the dialytic glucose load, amino acid solutions have been developed, but their biocompatibility and nutritive effects in children are uncertain. In the setting of malnutrition, especially in young children, the focus is on optimizing dietary intake, which may require tube feeding.

Multichamber PD bags separate the glucose at a very low pH from the buffer. This largely prevents the formation of toxic glucose degradation products (GDP) during sterilization and storage (Tables 1 and 2). Numerous experimental studies demonstrate untoward effects of GDP. Clinical trials suggest better preservation of peritoneal membrane function and of residual kidney function with low-GDP PD fluids (see Chap. 8). The unphysiological high concentrations of lactate may be circumvented by application of PD solutions containing bicarbonate buffer. A prospective comparison in children with a respective neutral pH, lactate-based solution suggested better preservation of the ultrafiltration capacity with a pure bicarbonate fluid [103]; experimental and ex vivo data suggest reduced peritoneal angiogenesis [32]. Additional benefits may be achieved with bicarbonate buffered fluids in patients with lactate acidosis, as present in case of sepsis and decompensated (inherited) metabolic diseases. The European Pediatric Dialysis Working Group recommends usage of the more expensive, low-GDP fluids whenever possible, and this has

Table 1 Composition of conventional, single-chamber PD solutions

	CAPD 2/3/4 17/18/19	**Dianeal PD 1, PD2#, PD4**	**Gambrosol 10/40**
Sodium (mmol/L)	134	132	132
Chloride (mmol/L)	102.5	102/96/95	96/95
Calcium (mmol/L)	1.25/1.75	1.75/1.75/1.25	1.75/1.35
Magnesium (mmol/L)	0.5	0.75/0.75/0.25	0.25
Glucose (%)	1.5/2.3/4.25	1.36/2.27/3.86	1.5/2.5/4.0
Osmolarity (mosmol/L)	356–509	344–486	353–492
Lactate (mmol/L)	35	35/40/40	40
pH	5.5	5.5	5.5
Formaldehyde (μmol/L)[a]	5.4 ± 0.4	6.8 ± 0.2	6.4 ± 0.5
3-DG (μmol/L)[a]	142 ± 0.8	167 ± 0.3	175 ± 4
3,4-DGE (μmol/L)[a]	16.2 ± 0.8	11.3 ± 0.5	13.1 ± 1.1

[a] At medium glucose concentration; 3-DG = 3-deoxyglucosone; 3,4-DGE = 3,4-dideoxyglucosone-3-ene. With permission from Schmitt et al. [100]

Table 2 Peritoneal equilibration test (PET) results as described by Warady et al. in a cross-sectional study in 95 pediatric patients on long-term PD (mean age 9.9±5.6 years) using 1100 ml/m2 dwell volume [126]. Findings may differ in incident patients, according to ethnicity and age

Patients	Type	4-h D/P creatinine	Characteristics	Measures to consider
10%	High	>0.77	Rapid solute transport absorption of glucose low UF dialytic loss of proteins? Low serum albumin?	Icodextrin modality switch with chronic use of 4% glucose concentrations?
53%	High average	0.65–0.77	Good solute transport moderate UF	APD, shorter and more frequent dwells needed Icodextrin for long dwell
31%	Average	0.51–0.64	Moderate solute transport good UF	APD (CAPD)
6%	Low	<0.51	Slow solute transport very good UF	Long dwells on APD, CAPD

meanwhile been realized in most European and numerous other countries.

Prescription of Peritoneal Dialysis

The child with kidney failure needs adequate replacement of kidney function. Adequate dialysis should be considered as the therapy that allows a nutritional state and growth in normal ranges, as well as a general well-being of the child. Volume overload and uremic status are the main complications that must be resolved, so peritoneal dialysis therapy must focus on obtaining adequate ultrafiltration and clearance of solutes. Each patient's prescription should be individualized according to the water and solute transport capacity of the peritoneal membrane as determined by the peritoneal equilibrium test (PET). Peritoneal dialysis therapy must be prescribed differently for patients who transfer solutes and glucose rapidly, as opposed to slow transporters. The dialysis regime administered should avoid excessive or insufficient solute clearance. The urea clearance applied in a determined time (T) over a solute distribution volume (V), the Kt/V, should therefore be evaluated together with the PET to adjust PD to the requirements of each patient.

Clinical Approach to the Peritoneal Transport

Two concepts have been recognized as important to prescribe dialysis therapy to patient's needs [125]. The first one corresponds to the dialysis dose as measured by urea Kt/V, and the second one evaluates the membrane transport capacity

Table a Composition of multichamber PD solutions

	BicaVera	Balance	Gambrosol trio 10/40	Physioneal 35/40	Extraneal (7.5% icodextrin)	Nutrineal (1.1% AS)
Sodium (mmol/L)	132	134	132*	132	132	132
Chloride (mmol/L)	104.5	100.5	96*	101/95	96	105
Calcium (mmol/L)	1.75	1.25/1.75	1.75/1.35*	1.75/1.25	1.75	1.25
Magnesium (mmol/L)	0.5	0.5	0.25*	0.25	0.25	0.25
Glucose (%)	1.5/2.3/ 4.25	1.5/2.3/ 4.25	1.5/2.5/3.9	1.36/2.27/ 3.86	0	0
Osmolarity (mosmol/L)[a]	358–511	358–511	356–483	344–484	284	365
Lactate (mmol/L)	0	35	40*	10/15	40	40
Bicarbonate (mmol/L)	34	0	0	25/25	0	0
pH	7.4	7.0	5.5–6.5[a]	7.4	5.5	6.7
Formaldehyde (μmol/L)*	<3.3	<3.3	<3.3	3.4 ± 0	3.6 ± 0.7	n.d.
3-DG* (μmol/L)	16.3 ± 0.2	17.6 ± 0.3	20.2 ± 2.4	93.3 ± 5.0	7.5 ± 0.4	<0.1
3,4 DGE* (μmol/L)	<2.4	<2.4	<2.4	14.3 ± 2.5	<2.4	n.d.

[a] Low to high glucose concentration; *at medium glucose concentration; n.d. = not done; 3-DG = 3-deoxyglucosone; 3,4-DGE = 3,4-dideoxyglucosone-3-ene. With permission from Schmitt et al. [100]

through the peritoneal equilibrium test. Although both concepts involve solute and water transport, urea Kt/V mainly refers to small-solute transport, because depuration mostly depends on the number of cycles and dwell volume. An inadequate PD regime that does not correspond to the PET result will, however, also be reflected by insufficient ultrafiltration and volume overload [40, 88, 125]. The evaluation of the optimal PD dose in clinical practice through tests like the mass transfer-area coefficient (MTAC) and measurement of the hydraulic conductance of the peritoneal membrane results are too complex for clinical routine. The simplicity of urea Kt/V and PET has been the main reason why they have replaced more complex mathematical calculation in clinical guidelines.

The Dialysis Dose, Kt/V Urea

The concepts of **urea Kt/V** and its equivalent, weekly creatinine clearance, were tested decades ago by the CANUSA study [17] and later by the prospective randomized ADEMEX study [82] and others [68]. Although both parameters have been considered to represent dialysis dose, urea removal is more related to the number of cycles and dwell volume, while creatinine clearance is more related to PD duration, because creatinine requires longer membrane transport times.

The Kt/V formula considers three parameters. The first of them is K, clearance (UV/P). K is multiplied by a dialysis time “t” of seven (days) and adjusted to the volume of distribution of urea, total body water “V.” In clinical practice, an easy way to calculate total body water is to multiply 0.6 times body weight (kg), since about 2/3 of the body weight is water. However, a more accurate equation for pediatrics should be used. According to the KDOQI guidelines, TBW should be calculated using the anthropometric formulas of Mellits and Cheek, later revisited by other authors [75]:

$$\mathrm{Kt/Vp\ (peritoneal)} = [\mathrm{Vol\ dial\ (L\ 24\ h)} \times \mathrm{BUN\ dial/BUN\ plasma}] \times 7/\mathrm{TBW}$$

$$\mathrm{Kt/Vr\ (residual)} = (\mathrm{Volume\ of\ urine\ (L\ 24\ h)} \times \mathrm{BUN\ urine/BUN\ plasma}) \times 7/\mathrm{TBW}$$

$$\mathrm{Kt/Vt\ (total)} = \mathrm{Kt/Vp} + \mathrm{Kt/Vr}$$

The CANUSA study evaluated the adequacy of dialysis as measured by the total weekly urea clearance (Kt/V) and total weekly creatinine clearance, finding an 8% decrease in relative risk (RR) of death for each 5 L/weekly of creatinine clearance, the same for each 0.1/week increase in total Kt/V. However, the main effect on patient survival was due to the residual kidney function instead the peritoneal small-solute clearance [6]. In the ADEMEX study, patients treated with a peritoneal urea Kt/V of 1.45–1.74 versus 1.94–2.24 over 2 years mortality was comparable, but RKF significantly impacted patient survival. Based on these and other experiences [80], the 2006 KDOQI guidelines [59] and the Canadian Society of Nephrology [12] recommend that the minimal delivered total dialysis dose (peritoneal and the kidney) should be at least 1.8 and 1.7, respectively, and weekly creatinine clearance >45/1.73 m^2 per week, with special reference to those patients without RKF, defined as a renal Kt/V < 0.1. The KDOQI guideline also suggests that for children, the recommended Kt/V should be at least the adult values. Likewise, the recent ISPD guideline suggests a total Kt/V (peritoneal and the kidney) of at least 1.7 for children [15].

Kt/V has been of help to prescribe the dialysis dose in clinical practice; however, urea is a low-molecular-weight solute, and its depuration is not parallel with other solutes and fluid removal. Therefore, the urea Kt/V should not be used as the only parameter of peritoneal dialysis adequacy [88]. In children, dialysis prescription should be individualized and, in addition to Kt/V assessment of dialysis adequacy, should include the overall clinical status of the patient. In view of the critical importance of controlling serum phosphate with regard to CKD-mineral and bone metabolism (MBD) and its implication in cardiovascular health, the ISPD recently suggested adjusting dialysis dose to optimize serum phosphorus [15].

As mentioned previously, the membrane transfer capacity should be evaluated in each patient through the peritoneal equilibrium test, which represents the capacity of the peritoneal membrane to transfer solutes and water during the dwell.

The Peritoneal Equilibrium Test

In 1987, Twardowski et al. [113] developed a standard test to assess the transport capacity of the peritoneum, the peritoneal equilibrium test (PET). It measures the speed which the concentration of a solute (creatinine) is balanced between the plasma and the dialysis solution by evaluating the ratio of creatinine in dialysate to creatinine in plasma (D/P creatinine) over 4 h. The appearance of creatinine in dialysate is usually measured at hours 0, 2, and 4 along with plasma creatinine at hour 2 (both in mg/dl). Since the initial dialysate creatinine concentration is zero, the creatinine ratio D_0/P_2 will be $= 0$ and will rise in the proportion that this solute is transferred from plasma to dialysate, reaching a maximum theoretical value $D_4/P_2 = 1$ representing a 100% transfer. In cases of low peritoneal transfer rate, the D_4/P_2 creatinine ratio remains low.

Since the velocity of peritoneal glucose uptake is relevant for ultrafiltration capacity, glucose concentration drop in the peritoneal fluid is measured at the same time points as creatinine and expressed as $D_{0\text{–}2\text{-}4}/D_0$. In addition to glucose uptake, it reflects the dilution of the dialysate by ultrafiltration. This includes the movement of free water, a process that predominates in the first hour, and the movement of water and solutes by convection, a process that predominates in the following hours of dialysis. Thus, the initial D_0/D_0 value will always be 1, decreasing later in direct proportion to the factors previously described, deducing that the lower the D_4/D_0 ratio, the greater the transfer capacity of water of the peritoneal membrane and the peritoneal glucose uptake is and vice versa.

From these two parameters, patients are classified into four categories, according to the mean value of the population ± 1 SD. The categories correspond to the following:

- High transporter
  ($D/P_{creatinine} >$ +1 SD of the mean value).
- High average
  ($D/P_{creatinine}$ between the mean and +1 SD of the mean value).

- Low average (D/$P_{creatinine}$ between the mean and −1 SD of the mean value).
- Low transporter (D/$P_{creatinine} < -1$ SD of the mean value).

These categories define the velocity of peritoneal transport, where a high transporter moves solutes and absorbs glucose relatively quickly, in opposite to a low transporter where more time is required to equilibrate plasma and dialysate compartments.

Different authors have reported different cutoff values, since the average of the population studied differs between centers and ethnicities. Cutoff values as obtained in 1996 by Warady et al. in a cross-sectional analysis in children on PD, relative frequency of transporter types, and potential clinical consequences are given in Table 2. In 2007, Warady et al. [123] reported cutoff values for these four categories in a shortened test (short PET) of 2 h. The clinical significance of the transport category is of great importance in clinical practice [125]. Fast transporters generally maintain good solute clearance, but the rapid drop in glucose in the dialysis fluid dissipates the osmotic gradient needed for ultrafiltration, with the risk of volume overload. Short dwell times, as applied in automated peritoneal dialysis (APD, NIPD), are appropriate in this type of patient. Conversely, a low transporter is characterized by a slow transport rate, achieving good ultrafiltration and solute clearance with long dwell times, e.g., 4–6 h as in a CAPD regimen. Short dwell cycles will interrupt ultrafiltration long before the peak of water transport has been reached and result in volume overload and low purification. If cycler PD is used (about hourly nocturnal dwells), long daytime cycles may be required for membranes with low-average transport capacity (CCPD). An inadequate dialysis prescription will lead to insufficient ultrafiltration rates resulting in volume overload and a lower than expected solute clearance (Table 2). Ultrafiltration failure, defined in adults as less than 400 mL in 4-h dwell (3.86% glucose) [46] and less than 150 ml/m^2 during a standard PET in children [124, 125], may ultimately develop with long-term PD and progressive peritoneal transformation (see Chap. 8).

Standardization of PET: Pre-PET Long Dwell

In the original description of the PET, the dwell time of the preceding exchange was approximately 8 h. During this time, the dialysate reaches equilibrium with the plasma. The pre-PET exchange is important because it influences the biochemical condition of the patient at time 0. Lilaj et al. [67] showed that the absence of a pre-PET dialysis cycle significantly altered the D/P values of low-molecular-weight solutes such as urea, creatinine, and proteins. While a long dwell exchange is easily performed in adult CAPD patients, pediatric patients are often on automated peritoneal dialysis, where an 8-h dwell is not suitable. Twardowski et al. [114] demonstrated that a dwell time between 3 and 8 h was acceptable for the preceding exchange dwell, followed by a two (short PET) or four (classic PET) dwell.

PET Protocol Following a 3–8 h dwell, the peritoneal cavity is thoroughly drained upon arrival at the dialysis center. A 2.5% dwell bag with Y-type connection is used to infuse 1100 ml/m^2 (maximum volume 2 liters) in 15 min, with the patient in a supine position and rotating from side to side to promote optimal mixing of the dialysis fluid. Dialysate samples are obtained at 0, 2, and 4 h of the test. To this end, 100–200 ml of dialysate is temporarily extracted (from 1000 to 2000 ml dwells), 5–10 ml samples are collected for creatinine and glucose measurement, and the remaining dialysate is reinfused. A blood sample is obtained after 2 h; complete dialysate drainage is performed after 4 h.

Standardization of Dwell Volume

As discussed previously, the volume of each dwell should meet the requirement of maximizing the peritoneal exchange surface, expressed as the area of pores available for diffusion (Ao/Δx), measured in $cm^2/1.73\ m^2$. Independently of age, the peritoneal membrane surface area is related to the body surface area, while expressed per kg body weight, the peritoneal membrane in infants has twice the surface area compared to adults. A volume calculated in ml/kg results in a smaller dialysate volume for the actual peritoneal exchange area and in faster solute exchange, reflected in the PET by a high D/P creatinine ratio and a low D/D_0 glucose ratio, generating an inaccurate "functional hyperpermeability" [43]. Therefore, the dwell volume should be calculated relative to body surface area.

The dwell volume should consider the resulting intraperitoneal pressure. A high intraperitoneal pressure (IPP) can cause pain and major complications such as hydrothorax, hernias, hydrocele, gastroesophageal reflux, and anorexia and ultimately lead to noncompliance and inadequate PD [41]. High dialysate glucose concentrations, organomegaly, high BMI, constipation, meteorism, abdominal pain, and conventional PD fluids increase intraperitoneal pressure. The majority of patients do not correctly perceive intraperitoneal pressure (IPP) during PD. Standardized measurement of IPP has therefore been suggested, and the details of procedure have been published [41]. The maximum tolerable intraperitoneal volume has been defined in adults as equivalent to an IPP of 18 cm H_2O, above which pain and a decrease in vital respiratory capacity have been reported. An observational study in 61 adult PD patients suggests an increased risk of enteric peritonitis with IPP above 14 cm H_2O [26]. It has therefore been suggested to limit IPP in children on nocturnal PD and above 2 years of age to 14 cm H_2O and to 8–10 cm H_2O in infants, since the latter have a higher risk of hernia and leakage. These IPP values correspond to a dwell volume of about 1200–1400 and 600–800 ml/m^2 BSA, respectively, which should probably not be exceeded. Prospective studies supporting these notions, however, have not yet been performed.

In case urgent PD is needed immediately after PD catheter insertion, initial fill volume should be about 10 ml/kg and can subsequently be increased according to individual needs and risks of leakage (presence of factors increasing intraperitoneal pressure). After that, a volume of 600–800 ml/m^2 in infants and 800–1400 ml/m^2 by dwell in pediatric age may be used as maintenance, always attending tolerance and intraperitoneal pressure.

The ultrafiltration capacity loss over the years leads to chronic volume overload which has been related to the poor cardiovascular outcome in this population. The classic PET is a reliable method to evaluate the peritoneal transport capacity regarding solute and water transport by convection and diffusion but gives no accurate information on ultrafiltration, as shown by the absence of correlation between 2-h creatinine D/P or glucose D/D with solute free water transport (FWT) and ultrafiltration. The PET shows a variability coefficient of $<10\%$ when assessing the transport of small solutes; however, this coefficient increases to 50% when UF is evaluated. The mini PET has therefore been introduced to evaluate the free water transport in dialyzed patients [64].

Free Water Transport: Mini PET

Ultrasmall pores correspond to aquaporin-1 and account for the movement of free water through the peritoneal membrane. In adults and children, during a hypertonic dwell, approximately 40%–50% of UF occurs through the ultrasmall pores as free water without solutes. La Milia et al. [63] proposed a test where a dwell of hypertonic solution (glucose 3.86%) is instilled, allowing measure of the peak of free water transport after 1 h of dwell time based on the osmotic effect of hypertonic dialysate, while sodium transport is minimal due to the low gradient of plasma-dialysate. As detailed previously, sodium sieving, or the decline in sodium in the dwell, is equivalent to the passage of free water from plasma to peritoneum. Only one group thus far has published data on sodium sieving in children [92], even though sodium sieving is clinically relevant. Short dwells with high glucose concentrations as frequently applied in APD remove more water than solutes and may therefore increase total body sodium content and thus thirst, a vicious circle, eventually resulting in high blood pressure.

Optimizing PD Prescription

Volume overload has been repeatedly shown to predict patient outcome. The majority of children on PD are salt and fluid overloaded. An optimized PD regimen requires adequate removal of sodium and water. Based on the fact that free water transport takes place mainly during the first hour of the dialysis cycle while solutes like urea and sodium move slower and mainly through diffusion and convective forces, i.e., relative solute clearance increases with dwell time and dwell volume, whereas net ultrafiltration rates decrease due to the dissipation of the osmotic gradient, Fischbach proposed a modified automated PD regimen combining short, small dwells and high glucose concentration with long and large dwells, the adapted APD [40]. The short, small dwells should favor free water transfer and may result in some hemoconcentration and dilution of the residual dialysate, potentially creating a higher solute gradient for the subsequent large and long dwells. This and the larger dwell volume increase in the peritoneal surface area recruited for exchange should promote solute (sodium) removal (Fig. 1). These assumptions were supported by a randomized, crossed over trial in 19 adult PD patients over two 45-day study periods. The standard PD study period comprised six cycles of 90 min, 2000 ml per cycle, and the adapted one consisted of two initial cycles, 45 min, 1500 ml, followed by three cycles of 3000 ml each, 150 min. Both groups received a similar total volume of 12,000 ml per dialysis session [39]. Both dialysis regimens were well tolerated, but the adapted APD regime resulted in significantly greater clearance of urea, creatinine, and phosphate; higher sodium removal and ultrafiltration; and improved blood pressure. Since then, this approach has been applied in an increasing number of patients, and personal communications are mostly positives, but further systematic studies are lacking, and criteria for individual prescriptions have not been established. Registries suggest a high variability in prescribed adapted APD regimens. The high intraperitoneal pressure created with the large dwells has to be carefully considered (at best measured in each patient), and associated risks of leaks and hernia should be counterbalanced against the potential benefits [44].

Taken together, better control of uremic toxins, especially phosphate, which is closely related to CKD-MBD and CVD, together with optimizing ultrafiltration and sodium removal, which are closely linked to volume overload and cardiovascular disease, is all the result of an appropriate PD prescription. The PD regime should be adjusted periodically in each patient to achieve these results, and surveillance through PET or mini PET, consideration of intraperitoneal pressure, and measurement of Kt/V should be applied routinely to achieve these goals.

The number of cycles overnight may vary according to residual kidney function and the required amount of ultrafiltration and solute removal during nightly intermittent PD (NIPD). One or two additional exchanges may be added during daytime in automated PD (APD), which is then called continuous cycling PD (CCPD). The single long daytime dwell is suited for icodextrin usage. Tidal PD may be useful, e.g., in patients with drainage problems. Cycles are performed over a residual volume; a complete drainage may be performed every 3–5 tidal cycles. Adapted APD uses the same total amount of PD fluid overnight as NIPD but varying dwell time and volume within one session.

Complications of PD

Infectious Complications

PD catheter-related infections include catheter exit site and tunnel infections and peritonitis. Although there is regional variation in infection rates among children on maintenance PD, recent data suggest that in general, rates are decreasing, with a drop in the annualized peritonitis rate among children enrolled in the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) registry from 0.79 episodes per patient year in children initiating PD between 1992 and 1996 to a rate of 0.44 in children initiating PD between 2007 and 2010 [80, 99]. Among children on PD enrolled in the IPPN between 2007 and 2018, the annualized rate of peritonitis was 0.44 [125]. The Standardizing Care to Improve Outcomes in Pediatric End-Stage Kidney Disease (SCOPE) Collaborative, a multicenter quality improvement collaborative in the United States, demonstrated a significant reduction in peritonitis rates by increasing implementation of a standardized PD follow-up care bundle, from a mean monthly rate of 0.63 episodes per patient year at the start of the collaborative to 0.42 episodes per patient years at 3 years post launch [79]. Despite these improvements, infection rates in children on PD remain higher than in adults, and national and international registries continue to reveal that PD catheter-related infections are a frequent and serious complication in children. Peritonitis is the most frequent cause for hospitalization in children on PD, and recurrent peritonitis is a leading contributor to PD technique failure [22, 23, 80, 97]. Infection is a leading cause of death in children on PD [22, 80, 109]. Fortunately, the ISPD has published guidelines specifically for the prevention and treatment of PD catheter-related infections in children [127]. Although early guidelines were largely opinion based, the most recent guidelines were significantly informed by prospective data collected on more than 500 episodes of PD-associated peritonitis in children through the International Pediatric Peritonitis Registry (IPPR), which was conducted with the goal of evaluating the safety and efficacy of the opinion-based ISPD guidelines [37, 99, 127].

Infection Prevention Given the significant morbidity and mortality associated with PD catheter-related infections, infection prevention should be a major focus of the care of the child on PD. Efforts to prevent PD-associated infections begin with PD catheter selection and placement. The types of PD catheter available for children is discussed previously, but in terms of infection prevention, a catheter with two subcutaneous cuffs, rather than one, and a downward or lateral orientation of the catheter as it emerges from the skin, rather than upward, have been associated with lower rates of peritonitis [66, 80, 104, 127, 135]. Infection prevention efforts during catheter placement also include the delivery of intravenous antibiotics prior to the surgical incision [107, 127].

Once the catheter is placed, efforts to optimize healing of the catheter tunnel and exit site, including catheter immobilization and minimizing the frequency of dressing changes, are important to minimize the risk for infection [86]. Delaying use of the PD catheter for dialysis until the exit site is healed will reduce the risk for leaking at the catheter exit site and the associated risk for infection, and data from SCOPE revealed that use of the catheter in the first 14 days after placement was associated with increased risk for early peritonitis [60, 87]. Regular cleansing with a sterile antiseptic solution followed by application of a topical antibiotic is recommended once the exit site is healed [86, 127].

The training of the child and/or caregivers to perform PD in the home should include education about infection prevention, including proper technique for hand hygiene, the aseptic technique for performing the dialysis procedure, and recognition of touch contamination and other breaks in aseptic technique [11, 127]. Education should also include corrective actions for breaks in aseptic technique, which may include treatment with antibiotics and/or transfer set change [127]. Early detection of and treatment for exit site and tunnel infection are important to minimize the risk for subsequent peritonitis [11, 127]. Retraining of caregivers after a peritonitis episode is encouraged [11, 127]. The standardized follow-up care bundle implemented by the SCOPE Collaborative requires that key aspects of hand hygiene, aseptic technique, and exit site care be reviewed with the child/caregiver and that the exit site be scored using an objective scoring tool at each visit in the PD clinic [1, 78, 98]. Compliance with this bundle has been associated with a significant reduction in peritonitis rates among the children on PD enrolled in that collaborative [79]. Additional infection prevention efforts include the provision of prophylactic antimicrobials with invasive procedures and antifungal prophylaxis whenever systemic antibiotics are prescribed [127].

Diagnosis and Treatment of Infection The diagnosis of peritonitis should be considered in any child on PD with abdominal pain and/or cloudy PD effluent, and the diagnosis is confirmed if the PD effluent white blood cell (WBC) count is greater than 100/mm^3 with at least 50% polymorphonuclear neutrophils (PMN) [127]. The PD effluent WBC count should be obtained from a dwell instilled for at least 1–2 h in patients on automated PD. When the effluent cell count is obtained after a shortened dwell, the presence of 50% or more PMN is highly suggestive of peritonitis when the clinical features of peritonitis are present, even if the total WBC count is below 100/mm^3 [127]. Current guidelines recommend that empiric antibiotic treatment be initiated as soon as the diagnosis of peritonitis is considered. Empiric antibiotic coverage should include both gram-positive and gram-negative organisms [127]. In children on PD, gram-positive organisms are the most common causes of peritonitis. Significant regional variability in causative organisms and antibiotic susceptibility have been demonstrated in both IPPR and IPPN, and therefore, local or regional culture results and antibiograms should guide empiric therapy [99, 125]. Intraperitoneal delivery of antibiotics is preferable, typically as continuous therapy with a loading dose which is allowed to dwell for 3–6 h, followed by a lower maintenance dose [127]. The loading and maintenance doses of the most commonly used medications to treat PD-associated peritonitis in children are shown in Table 4 [127].

Current guidelines suggest that monotherapy with cefepime may be considered for empiric treatment for PD-associated peritonitis in children [127]. If cefepime is not available, a first-generation cephalosporin or a glycopeptide (vancomycin or teicoplanin) combined with ceftazidime or an aminoglycoside may be considered [127]. Empiric antibiotics should be modified once culture results are available. Treatment duration should be guided by the causative organism and the clinical course of the child and typically ranges from 14 to 21 days. Fungal prophylaxis is recommended during treatment for bacterial peritonitis [127]. Early identification and aggressive treatment are vital to minimize the risk of peritonitis-related morbidity, including peritoneal membrane failure, and mortality. Peritoneal dialysis catheter removal may be necessary to eliminate infection and minimize injury to the peritoneal membrane, particularly in the setting of refractory bacterial peritonitis, when peritonitis and exit site and/or tunnel infection occur simultaneously with the same organism. Catheter removal is nearly always required in the setting of fungal peritonitis [127].

Purulent drainage at the PD catheter exit site is diagnostic of an infection; however, infection should also be considered when pericatheter swelling, erythema, and/or tenderness is noted, with or without purulent drainage [111, 127]. A PD catheter tunnel infection is diagnosed when erythema, edema, and/or tenderness extends along the catheter's subcutaneous tunnel, with or without the presence of purulent drainage from the exit site [111, 127]. Use of ultrasound may help verify extension of the infection to the tunnel. Exit site and tunnel infections may be caused by many different organisms, including normal skin flora such as corynebacteria [84]. *Staphylococcus aureus* is the most common causative organism, but gram-negative organisms, including *Pseudomonas* species, are increasingly identified in exit site and tunnel infections [84, 85, 108].

Exit site and tunnel infections are typically treated with oral antibiotics, with culture results and antibiotic susceptibilities guiding antibiotic selection [127]. Tunnel infections may require intraperitoneal or intravenous antibiotic therapy if there are signs of severe infection and/or a history of infection with *S. aureus* or *Pseudomonas aeruginosa*. Treatment duration should be guided by evidence of clinical improvement and is continued for at least 7 days after complete resolution of the signs/symptoms that prompted diagnosis [127]. A typical treatment duration is 2 weeks for organisms other than *S. aureus* or *P. aeruginosa*, for which treatment is extended to at least 3 weeks. If complete resolution of the signs/symptoms of infection is not achieved during this time frame or the child on PD develops peritonitis due to the same organism, catheter removal is recommended [127].

Noninfectious Complications

Although infection remains the most common complication of PD in children, noninfectious complications are also a significant problem and can be associated with significant morbidity, including the need to terminate PD [14, 89]. Noninfectious PD complications can be divided into catheter-related complications and complications related to the dialysis procedure [71].

Catheter-Related Complications Catheter-related complications include dialysate leak at the catheter exit site and mechanical catheter dysfunction resulting in inadequate inflow or outflow of dialysate during the dialysis procedure. As mentioned previously, early catheter leak can be seen when the PD catheter is used for dialysis before the catheter tunnel/exit site has healed, and delaying use of the catheter when possible is recommended to minimize this risk [60, 87, 127]. Mechanical dysfunction can occur due to kinking of the catheter, obstruction of the catheter lumen by fibrin or clot, occlusion of the catheter perforations by omentum, and migration of the catheter tip out of the pelvis [71]. Instillation of heparin or tissue plasminogen activator may restore catheter function when obstruction is due to fibrin or clot [135]. Both catheter leak and mechanical dysfunction due to omental occlusion and migration of the catheter may require catheter revision to restore function. Data from the IPDN revealed that among 452 catheter revisions reported to that registry, 60% were performed for mechanical dysfunction and 6% for catheter leak [14]. Constipation may also cause catheter dysfunction by encouraging catheter tip migration as well as external compression of the lumen by bowel [71]. Therefore, children on PD should be regularly monitored for constipation, and bowel regimens to minimize the risk for constipation should be prescribed. Although strictly speaking not a complication, discomfort with inflow and outflow should be anticipated. Discomfort with inflow can be associated with the unphysiological composition of dialysate fluid and "shear" stress, which may abate with time. Outflow discomfort may be related to adherence of the catheter to intraperitoneal structures, especially with small dwell volumes.

Complications Related to the Dialysis Procedure Complications related to the dialysis procedure can be divided into three groups by causation: 1) increased intraperitoneal pressure due to instillation of dialysis fluid into the abdomen, 2) transfer of solute during the dialysis procedure (i.e., metabolic or electrolyte abnormalities), and 3) exposure of the peritoneum to dialysis fluid [71]. The last group is discussed in section "PD Membrane Transformation/EPS" of this chapter.

As mentioned in section "Prescription of Peritoneal Dialysis," adequate exposure of the peritoneal membrane to dialysis fluid is essential for effective solute and water transfer which, in turn, is optimized by increasing the dialysate fill volume. However, increasing fill volume results in increasing intraperitoneal pressure (IPP) [42]. Elevated IPP not only can lead to patient discomfort and intolerance of the dialysis procedure but also may increase the risk of dialysate leaks, even after the catheter exit site has healed [27]. These leaks may present with accumulation of fluid in the subcutaneous tissue, weight gain, and peripheral and/or genital edema. Late leaks may resolve with reduction in fill volume, avoiding a daytime fill, or temporary cessation of PD [27]. Gastroesophageal reflux may also be exacerbated by IPP, and in addition to the typical symptoms associated with reflux, presenting symptoms may include back or abdominal pain [71]. Therapeutic maneuvers include reducing fill volume, particularly during the day. Another complication related to IPP is hernias, which are reported to occur in 6%–8% of adult PD patients [65]. Higher rates are reported in children on PD, ranging from 8% to 57%, and are inversely proportional to age [4, 51, 65]. Areas of anatomic weakness including the umbilicus, linea alba, and inguinal canal, with or without a patent processus vaginalis, and surgical incision sites are at particular risk for hernia development. Given the high prevalence of a patent processus vaginalis in infants and toddlers, it is not surprising that inguinal hernias are common, occurring in over 50% of children starting PD under the age of 2 years in one case series [65]. Accordingly, some surgeons may perform closure of the processus vaginalis at the time of PD catheter insertion in this age group. Efforts to reduce IPP may allow small hernias to be followed, with monitoring for incarceration. However, many hernias in children on PD ultimately require surgical repair.

Another complication related to IPP is hydrothorax, which occurs when fluid enters the pleural space by way of a peritoneal pleural leak, presumably at the site of diaphragmatic defect, typically on the right [24, 110]. Presenting symptoms of hydrothorax include shortness of breath and chest discomfort. Once transudative effusion in the setting of fluid overload is ruled out, the diagnosis is confirmed with measurement of glucose in the fluid obtained by way of thoracocentesis, with an elevated pleural fluid glucose relative to serum glucose verifying peritoneal dialysate as the origin of the fluid. Transient cessation of PD may allow closure of the diaphragmatic defect, and chemical pleurodesis with tetracycline, talc, or autologous blood may be attempted if cessation of PD is not successful [24, 110]. Other therapeutic options include thoracoscopic pleurodesis and thoracoscopic or open diaphragmatic repair [24, 110]. Pericardio-peritoneal leak has also been reported in children on PD and typically presents with tachypnea. As with pleuroperitoneal leak, cessation of PD is usually required [30].

Complications Related to Transfer of Solutes during the Dialysis Procedure The transfer of solutes and water between plasma and dialysate is the therapeutic goal of the PD procedure. However, precise control of solute transfer is not possible, and resultant electrolyte and metabolic derangements should be anticipated and monitored for. Hypokalemia is the most common electrolyte derangement, as standard PD fluid does not contain potassium, and is particularly common in infants and young children who are exclusively fed low-potassium formula or breast milk. In those infants and children, modification of the formula and/or potassium supplementation may be required.

Hypermagnesemia is relatively common in children with chronic kidney disease (CKD) due to reduced clearance of magnesium by the kidney. Magnesium concentrations in commercially available dialysis solutions for PD range from 0.25 to 0.75 mmol/L. Elevated magnesium levels are typically seen with use of solutions containing 0.75 mmol/L magnesium, while use of solutions containing both 0.5 and 0.25 mmol/L magnesium has been associated with hypomagnesemia in adult patients [130, 131]. Inverse correlations between serum magnesium levels and parathyroid hormone (PTH) and fibroblast growth factor 23 have been demonstrated in adults on dialysis, raising concern for increased risk for adynamic bone disease with hypermagnesemia and osteitis fibrosa with hypomagnesemia [54, 81, 130, 131]. Given the lack of data available on magnesium homeostasis in children on PD, current recommendations suggest choosing a solution that allows maintenance of a high normal serum magnesium, i.e., 0.9–1.0 mmol/L, in this population [100].

Hyperglycemia, hyperinsulinemia, and dyslipidemia, known contributors to cardiovascular disease, are unfortunately present in children at the early stages of CKD. Hyperlipidemia was noted in 45% of children enrolled in the Chronic Kidney Disease in Children (CKiD) study, while 4% had hyperglycemia and 9% and 19% had hyperinsulinemia and insulin resistance, respectively [93, 134]. The complex pathophysiological mechanisms contributing to disturbances in glucose and lipid metabolism in CKD are beyond the scope of this chapter; however, it is important to recognize that exposure to glucose-containing dialysis solutions represents a substantial glucose load for children on PD which, in turn, may induce insulin resistance and an atherogenic lipid profile [10, 16, 28]. These findings reinforce recommendations to minimize exposure to glucose by using the lowest dextrose concentration possible, with the addition of icodextrin as required to maintain euvolemia [100]. Given the lack of data on safety and efficacy of pharmacologic agents to treat dyslipidemias in children, Kidney Disease: Improving Global Outcomes (KDIGO) guidelines suggest that statins or statin/ezetimibe combination not be initiated in children less than 18 years of age with CKD, including those treated with chronic dialysis [122]. Therefore, lifestyle modifications including nutrition and dietary counseling are the primary therapeutic intervention for dyslipidemia [122] (Table 3).

Table 3 Dosing recommendations for the treatment of peritonitis

	Continuous therapy[a]		
	Loading dose	Maintenance dose	Intermittent therapy
Aminoglycosides[b]			
Gentamicin	8 mg/L	4 mg/L	
Netilmicin	8 mg/L	4 mg/L	Anuric: 0.6 mg/kg Non-anuric: 0.75 mg/kg
Tobramycin	8 mg/L	4 mg/L	
Amikacin	25 mg/L	12 mg/L	
Cephalosporins			
Cefazolin	500 mg/L	125 mg/L	20 mg/kg
Cefepime	500 mg/L	125 mg/L	15 mg/kg
Cefotaxime	500 mg/L	250 mg/L	30 mg/kg
Ceftazidime	500 mg/L	125 mg/L	20 mg/kg
Glycopeptides[c]			
Vancomycin	1000 mg/L	25 mg/L	30 mg/kg; repeat dosing 15 mg/kg q 3–5 days
Teicoplanin	400 mg/L	20 mg/L	15 mg/kg q 5–7 days
Penicillins[b]			
Ampicillin	–	125 mg/L	–
Quinolones			
Ciprofloxacin	50 mg/L	25 mg/L	–
Others			
Aztreonam	1000 mg/L	250 mg/L	–
Clindamycin	300 mg/L	150 mg/L	–
Imipenem/cilastatin	250 mg/L	50 mg/L	–
Linezolid (oral)	<5 years: 30 mg/kg/day divided TID; 5–11 years: 20 mg/kg/day divided BID; ≥12 years: 600 mg/dose BID		
Metronidazole (oral)	30 mg/kg/day divided TID (max daily dose 1.2 g)		
Rifampin (oral)	10–20 mg/kg/day divided BID (max daily dose 600 mg)		
Antifungals			
Fluconazole	6–12 mg/kg **IP**, **IV,** or **PO** q 24–48 h (max daily dose 400 mg)		
Caspofungin	IV only: Initial dose 70 mg/m^2 on day 1 (max daily dose 70 mg); subsequent dosing 50 mg/m^2 daily (max daily dose 50 mg)		

Used with permission from Warady et al. [127]

Administration should be via intraperitoneal route unless specified otherwise

Intermittent doses should be applied once daily unless specified otherwise

[a] For continuous therapy, the exchange with the loading dose of antibiotics should dwell for 3–6 h, followed by the use of the maintenance dose for all subsequent exchanges

[b] Aminoglycosides and penicillins should not be mixed in dialysis fluid because of the potential for inactivation

[c] Accelerated glycopeptide elimination may occur in patients with residual renal function. If intermittent therapy is used in this setting, the second dose of antibiotic should be time-based on a blood level obtained 2–4 days after the initial dose. Redosing should occur when the blood level is <15 mg/L for vancomycin or 8 mg/L for teicoplanin. Intermittent therapy is not recommended for patients with residual renal function unless serum drug levels can be monitored in a timely manner

PD Membrane Transformation/EPS

Next to achievement of sufficient solute and water removal, preservation of peritoneal membrane function is essential for children requiring long-term PD, in particular in infants yet too small for transplantation and children returning to dialysis after kidney transplant failure. The main risk factors for loss of peritoneal membrane function are the unphysiological composition of PD fluids and severe, persistent, and/or relapsing peritoneal infections.

Single-chamber PD fluids have an acidic pH of 5.5, contain high concentrations of lactate, glucose, and glucose degradation products (GDP), the latter being formed during sterilization and prolonged storage (see Table 1). Studies in adults reveal these fluids result in progressive loss of the mesothelial cell layer, fibrosis and vasculopathy [132], and ultimately in PD failure. The frequency of PD termination due to ultrafiltration failure increases with the time of treatment, in adults from 14% in the first 5 years to 33% thereafter [52]. A key pathophysiological mechanism is transition of mesothelial cells to a mesenchymal phenotype (MMT) and migration of these cells into the submesothelial space, where they secrete numerous proinflammatory and profibrotic cytokines [70].

Double-chamber PD fluids separate glucose at a pH of around 3 from the electrolytes and the buffer, which can either be lactate, bicarbonate, or a mixture of both. GDP formation is largely prevented (see Table 2). Numerous experimental studies demonstrate less toxicity and preserved peritoneal immune response. Peritoneal tissue studies in 172 children, however, demonstrated major membrane transformation with angiogenesis, inflammatory cell invasion, MMT, and respective cytokine induction within few months of PD, and progressive fibrosis with long-term PD [95]. Associated vasculopathy is complement mediated [7]. The largest randomized clinical trial, the balANZ trial, comparing low- and high-GDP fluid PD in 185 patients over 2 years demonstrated lower UF rates and higher solute transport rates during the first year with low-GDP fluids, i.e., a high transporter status, but not thereafter. In patients treated with low-GDP fluids, peritoneal transport rate remained largely stable but increased significantly with high-GDP fluid usage; ultrafiltration rates declined [57, 58]. The trial furthermore suggested a lower incidence and severity of peritonitis with low-GDP exposure; time to anuria was reduced [57, 58]. Other RCTs reconfirmed superior preservation of residual kidney function, altogether reaching adequate power in a respective meta-analysis [53, 58]. Residual kidney function is associated with better survival, lower morbidity, and greater quality of life.

No systematic studies have been performed on the impact of icodextrin-based fluids on peritoneal tissue histology and inflammation. Clinical trials replacing the glucose-based PD solution by icodextrin during the single long dwell, however, demonstrated significantly improved ultrafiltration rates and reduced episodes of fluid overload in adults and a decrease in mortality by 50% compared to PD regimen using glucose-based fluids only. The lower peritoneal glucose absorption with icodextrin-containing PD, however, does not translate in significantly improved systemic glucose homeostasis [49]. Icodextrin may be used especially in children with fluid overload.

Peritonitis episodes may aggravate peritoneal membrane transformation, especially when they are severe and recurrent. In a retrospective study in children, peritonitis caused by *Pseudomonas aeruginosa* or alpha streptococcal organisms was an independent predictor of membrane failure, defined as severe loss of UF capacity [2]. A study comparing 36 children who never had an episode of peritonitis with 57 children with a history of one or more episodes of peritonitis [3] and a longitudinal follow-up study of 26 children suggested higher transport of glucose and creatinine in children with previous peritonitis episodes; all patients were treated with acidic high-GDP fluids. The power of such analyses is limited. Recent studies in children on low-GDP fluids indicate that adequately and successfully treated peritonitis episodes do not have a major impact on peritoneal membrane histology and inflammatory cell and cytokine profile [8]. The GLOBAL Fluid Study suggests an increase in the transporter status with peritonitis episodes together with acidic PD fluids, with high-GDP content, but not when low-GDP fluids are used [34].

In cases where the smoldering inflammatory and transformational process induced by peritonitis episodes and PD fluid toxicity continues, encapsulating peritoneal sclerosis (EPS) eventually develops. EPS is a life-threatening condition reported in 0.7%–3.3% of adult and 1.5%–1.9% of pediatric PD cohorts [105, 118]. Initial clinical symptoms usually comprise abdominal pain and small bowel dysfunction and progress, in its severest form, to complete intestinal obstruction as the bowel becomes trapped in the sclerotic peritoneum. The presence of peritoneal calcifications on abdominal computed tomography scan in association with ultrafiltration failure is highly suggestive of EPS. A European survey in 14 pediatric dialysis centers demonstrated a higher PD vintage of 5.9 (1.6–10.2) years and a higher peritonitis rate than in non-EPS patients. The median interval between UF failure and bowel obstruction was 3 months. In one out of four children, EPS was diagnosed after PD has already been discontinued, i.e., on HD or after renal transplantation. Treatment is debated and usually consists of enterolysis, immunosuppression, or tamoxifen [55]. Mortality was reported to be 35%–69% in adults [5] and 14% in children [105]. Clinical observations suggest a lower incidence of EPS with usage of neutral pH, low-GDP fluids, but respective data from prospective studies are lacking.

Remote Patient Monitoring (RPM)

Home PD requires significant medical and technical knowledge and encumbers families with major responsibility, preventing PD treatment in some families and resulting in early PD technique failures in others. Nonadherence with the prescribed regime is common. In Kansas City, 45% of 51 children exhibited some nonadherence to prescribed PD regimen [25]. One important strategy to improve the quality of care in PD is increasing surveillance and support of the families at home. Cyclers introduced at the beginning of the twenty-first century provide integrated digital card systems for retrospective insight into PD performance, complications, and adherence. The latest generations of PD cycler are now equipped with remote monitoring technology that allows for automated online data transfer of the PD regime, ultrafiltration volumes, and alarms, together with manually entered data such as body weight, blood pressure, and personal comments. This offers real-time remote patient monitoring options by far exceeding surveillance by phone calls, e-mails, and fax [121].

For implementation of RPM, a step wise process is needed (Table 4). Trained persons of the medical team obtain access to their patients' data collected online, which at best should be integrated in existing electronic health records and possibly be amended by videoconferencing functions. Close interaction with trained families is required (Fig. 1); alignment with regional and national laws and regulations has to be verified. Repeated and systematic analysis of the interaction process should improve the RPM process, and impact on current standards of care, e.g., may reduce the frequency of center follow-ups. Other benefits are timely recognition of PD-associated problems, such as catheter dysfunction and peritonitis and inadequate treatment performance, e.g., with regard to PD fluid turnover and ultrafiltration. Immediate adaptation of the treatment can be realized. Due to automated data generation, the burden of collecting and communicating treatment data is reduced. Blood pressure and body weight, however, still require manual entry. By sharing medical responsibility, RPM should reduce the overall burden perceived by the families and increase the families' confidence. The interactive communication should improve individual knowledge and promote the use of PD. Education features may be implemented, too (Fig. 2).

Grade 1A evidence in favor of RPM has been obtained in adult patients with heart failure. In these patients, RPM reduced hospitalizations and mortality, while the impact of mobile phone-based monitoring and videoconferencing remained uncertain [9]. Published RPM experience in pediatric PD is limited. RPM appears useful in

Table 4 Implementation steps for remote patient monitoring (RPM)

Choose device and parameters to monitor; decide on data monitoring and action process
Verify alignment with law and regulations
Train doctors and nurses, followed by patients and carers
Set individual patient alarm margins and alerts
Start RPM
Repeatedly review data sets, alerts, and decisions taken based on RPM
Assess patient perception and adherence to RPM
Reconsider individual and center RPM settings
Systematically evaluate RPM process, and reconsider standards of clinical practice

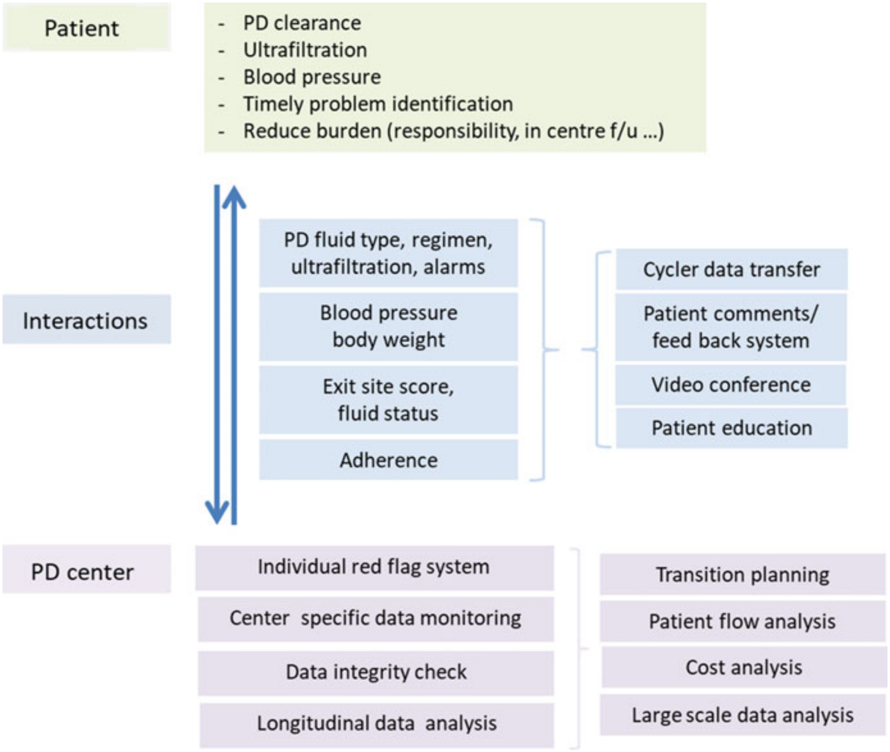
Patient
- PD clearance
- Ultrafiltration
- Blood pressure
- Timely problem identification
- Reduce burden (responsibility, in centre f/u ...)
Interactions
PD fluid type, regimen, ultrafiltration, alarms
Blood pressure body weight
Exit site score, fluid status
Adherence
Cycler data transfer
Patient comments/ feed back system
Video conference
Patient education
PD center
Individual red flag system
Center specific data monitoring
Data integrity check
Longitudinal data analysis
Transition planning
Patient flow analysis
Cost analysis
Large scale data analysis

Fig. 2 Schematic illustration of advanced remote patient management with data transfer between the patient and the family at home (green squares) and the dialysis center (purple squares) and respective interactions and possible tools (blue squares)

detecting and solving clinical and technical problems of automated PD, reduces the number of shortened PD treatments [31], and improves fluid status [21]. A prospective study on RPM including videoconferencing in 25 adult PD patients for a total of 200 RPM months and comprising 172 teleconsultations demonstrated reduced emergency room visits and hospitalizations; the associated costs were lower as compared to 32 non-remote managed patients, of whom, however, several had refused RPM [47]. The decline in patient contacts for technical and medical issues during the mean follow-up of one year may reflect RPM-related training effects and improved reassurance of both sides due to the higher degree of surveillance.

Of note, the benefits of monitoring numerous parameters have to be balanced against the risk of data overflow and require standardized RPM procedures within the center and careful communication with the families. Families need to be informed on how often and by whom the data is reviewed. The data surveillance and interventions should be feasible within clinical routine. Overreliance in RPM may reduce situational awareness and personal communication in case of acute problems. The monitoring functions require repeated, patient-specific margin settings and respective alarm signals. Potential technical pitfalls such as false readings must be considered. Thus, critical review of the pursued versus actual therapeutic success is essential at regular intervals during conventional face to face meetings (Table 5). Setting rigorous alarm systems in RPM may result in unnecessary, frequent perturbations of domestic ambiance and possibly in mental and cognitive disconnection with the alarms. Conversely, liberalizing alarm limits may not sufficiently alert families and clinicians to critical scenarios and thus result in potential patient harm. At present, RPM should be considered a beneficial adjunct, but cannot replace face to face communication. Otherwise nonverbal signs may be missed, e.g., in families not coping with the various demands. Likewise, continuous online data assessment may be perceived by some families as inappropriate surveillance and violation of privacy; the families should have the right to discontinue online data transfer times. Overall RPM acceptance, however, is good; the benefits of being supported at home obviously predominate over perceived disadvantages in the majority of families. And reports from pediatric dialysis centers are mostly positive.

Outcomes

Kidney transplantation is the treatment of choice for the vast majority of children with kidney failure. When preemptive transplantation is not possible, dialysis is typically viewed as a bridge or temporary treatment. The primary outcome of interest for children who do require dialysis, therefore, is time to kidney transplantation. Factors that influence access to and timing of kidney transplantation in children are covered is Chap. x.

A PD treatment-related outcome is technique failure or the requirement to discontinue PD due to the loss of a functioning peritoneal membrane. Among children on dialysis enrolled in NAPRTCS, 10% of dialysis terminations were due to inadequate ultrafiltration or solute clearance [80, 129]. In a recent analysis of data from the IPPN, among more than 2400 children on PD with over 3000 patient years of follow-up, 32 experienced technique failure [14]. A history of previous PD catheter revision was associated with a higher risk for PD failure [14].

Although mortality rates for children on dialysis are lower than those seen in adults and recent data suggest improved survival in more recent eras, death rates for children with kidney failure remain unacceptably high [72, 74, 109, 129]. In a recent analysis of data from Europe, the overall mortality rate was 28 deaths per 1000 patient years during the first 5 years of dialysis [22]. Mortality rates are highest in the first year of dialysis and among children under the age of 5 years at dialysis initiation [22]. In this study, children who initiated PD had a lower mortality risk than those on HD, particularly among those older than 5 years of age at dialysis initiation [22]. However, other studies have not demonstrated significant difference in mortality by dialysis modality, and it is recognized that selection bias may be a significant contributor to differences in survival reported in children on PD and HD [119, 129]. Young age has repeatedly been associated with increased risk for mortality among children on PD, with the highest death rates reported in children who initiate dialysis in infancy, although recent analyses suggest improved survival in the infants on PD [18, 80, 94, 109, 116, 129]. The presence of comorbidities and oliguria has both been associated with increased risk of death, particularly in infants on PD [18, 33, 77]. Black race is associated with increased risk for mortality among children on dialysis, which may at least in part be related to difference in access to transplantation [62, 74]. Infection and cardiovascular disease have repeatedly been shown to be the leading cause of death among children on dialysis [22, 74, 119, 129].

Outlook

Neutral pH, low-GDP and icodextrin PD fluids are increasingly used and should further improve PD patient outcome. Novel PD fluid prototypes are being developed. In a recent clinical phase 3 trial, addition of alanylglutamine to neutral pH, low-GDP fluid partially improved small-solute clearance, reduced peritoneal protein loss, improved biomarkers of peritoneal membrane integrity and immune competence, and reduced a marker of systemic inflammation as compared to unsupplemented PD fluid [120]. Current research is focused on novel osmotic compounds with reduced toxicity and lower resorption rates as compared to glucose. Future PD regime should allow for a more refined, individual adaptation of electrolyte and buffer supply to optimize mineral and acid-base balance in order to reduce CKD-MBD and cardiovascular disease. Likewise, advanced, comprehensive, and remote patient monitoring, ideally in a network of digital devices, e.g., including blood pressure and body weight monitoring and videoconferencing, should increase recruitment of PD patients and their families and improve education, quality of life, and patient outcome. On a larger scale, the accumulating treatment data should provide significant information on technical shortcomings, PD efficacy, and complications and may allow for refined individual prognostic modelling. The human element of medicine, on the other hand, must not be lost of sight when implementing the digitally enhanced care pathways. Critical and comprehensive evaluation is mandatory in order to improve our ultimate targets, as well- defined by the Song-PD initiative [69].

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Chronic Hemodialysis in Children

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Abstract

Conventional hemodialysis (HD) is a widely used kidney replacement therapy for children with end-stage kidney disease. HD is based on diffusive transport of solutes across a semipermeable membrane and is effective in removing small uremic retention solutes such as urea, correcting electrolyte abnormalities and restoring fluid balance. The addition of convective therapies with hemodiafiltration (HDF) allows the removal of larger uremic retention solutes, such as β_2-microglobulin and inflammatory cytokines. Extended hours of hemodialysis, best achieved through home hemodialysis programs, achieves optimal clearances without the hemodynamic strain of conventional HD. Despite technical advances, children on dialysis continue to experience high rates of morbidity and mortality compared to the general pediatric population.

In this chapter we discuss the principles, technique and practical aspects of performing conventional HD and intensified dialysis techniques, including HDF and home hemodialysis, in children, and relevant pediatric and adult studies to support these practices. We outline commonly encountered complications of HD and strategies for their prevention and treatment. Vascular access, often called the “Achilles’ heel” of dialysis, is discussed at length, comparing the relative merits of central venous lines and arteriovenous fistulas. Finally, we suggest future directions for children treated with HD including the benefits of intensified hemodialysis options.

Keywords

Hemodialysis (HD) · Hemodiafiltration (HDF) · Home hemodialysis · Diffusion · Convection · Arteriovenous fistula (AVF) · Central venous line (CVL) · Dialyzer · Intradialytic hypotension

Introduction

Hemodialysis (HD) is the most efficient dialysis modality. Advances in technology have brought hemodialysis from the rotating drum system first devised by Kolff [1] to a sophisticated, highly automated method applicable to patients from infancy to adulthood, delivered in multiple settings including patients' homes. With this advancement comes complexity requiring knowledge and thoughtful collaboration between physicians and dialysis staff to assure safe and effective patient care. Multiple components – vascular access, dialyzer, dialysis fluid proportioning system, ultrafiltration controller, and safety mechanisms – must work in harmony to provide a successful hemodialysis session. The dialysis team must leverage their expertise and clinical judgment to develop an appropriate hemodialysis prescription and deliver the therapy to the patient while monitoring closely for possible complications.

Epidemiology of Pediatric Hemodialysis

The United States Renal Data System (USRDS) 2019 Annual Data Report [2] demonstrates that 51.3% of incident patients with end-stage kidney disease (ESKD) in the USA aged 21 years or younger (677 of 1319) begin their ESKD care receiving HD, with the remainder split between peritoneal dialysis (27.8%) and kidney transplant (20.8%). By comparison, prevalence data within the same cohort show 73.3% undergoing kidney transplantation with only 16.6% receiving HD, which comprised 61.8% of children receiving maintenance dialysis. Distributions vary further depending on the age of the pediatric patient, with HD use being less common in younger patients. In the United Kingdom, the UK Renal Registry 21st Annual Report [3] indicates 966 prevalent patients age 18 years or younger at the end of 2017 requiring kidney replacement therapy (KRT); 217 of these patients were on maintenance dialysis with 120 (55.3%) on HD. The European Society for Pediatric Nephrology – European Renal Association Registry reports that in children aged <15 years from 37 European countries approximately 5.5 per million age-related population start KRT annually. Unlike trends in the USA, the majority of incident pediatric ESKD patients in Europe started KRT with peritoneal dialysis (47%), followed by hemodialysis (33%), and preemptive transplantation (20%) [4].

Dialysis Initiation

The optimal time to start chronic dialysis remains controversial. A recent Kidney Disease Improving Global Outcomes (KDIGO) guideline suggests that, in the absence of symptoms, there is no specific estimated glomerular filtration rate (eGFR) for initiation of dialysis and current data do not support preemptive dialysis initiation [5]. The IDEAL study, the first randomized controlled trial on dialysis initiation either in early or late CKD stage 5 (CKD5) in adults, showed that early initiation of dialysis (at an eGFR of 10–14 ml/min/1.73 m^2) in patients with CKD5 was not associated with an improvement in survival or clinical outcomes compared to an elective delayed start (an eGFR of 5–7 ml/min/1.73 m^2) [6]. IDEAL showed that with careful clinical management, dialysis may be delayed until either the eGFR drops below 7 ml/min/1.73 m^2 or traditional clinical indicators for the initiation of dialysis are present [6]. Although the Kidney Disease Outcomes Quality Initiative (KDOQI) guideline published in 2006 suggested that dialysis should be considered when eGFR falls to <14 ml/min/1.73 m^2 and recommended when eGFR falls further to <8 ml/min/1.73 m^2 [7], three recent national and international guideline bodies clearly indicate that in the absence of symptoms there is no benefit from starting dialysis early [5, 8, 9]. The UK National Institute for Health and Care Excellence (NICE) [8], the Canadian Society of Nephrology [9], and the 2015 KDOQI guideline for HD adequacy [10] have all suggested that dialysis should be initiated at an eGFR of 5 to 7 ml/min/1.73 m^2 if there are no uncontrollable uremic symptoms, biochemical abnormalities, or fluid overload.

In children, three studies from the USRDS and the European Society of Pediatric Nephrology (ESPN) registry [11–13] also conclude that there is no benefit from starting dialysis early. Taken together, these registries have examined the association between eGFR at initiation of chronic dialysis and clinical outcomes in over 21,000 children. Of almost 10,000 incident dialysis patients aged 1 to 17 years in the USRDS registry (1995–2016), a higher eGFR at the start of dialysis was associated with an increased risk of death. Mortality risk increased in those starting dialysis with eGFRs <5 and ≥ 12 ml/min/1.73 m^2, with a greater risk in patients 6 years and older [12]. A further study from the USRDS assessed eGFR in the range of 5–12 ml/min/1.73 m^2, in which most CKD5 patients start dialysis; the mortality risk was 36% higher for those with eGFR >10 ml/min/1.73 m^2 compared with those with lower eGFR at dialysis initiation [13]. An observational, retrospective cohort study of nearly 3000 patients aged <18 years from the ESPN registry examined two populations of children who started dialysis with an eGFR above or below 8 ml/min/1.73 m^2 [11] and found that mortality did not differ between the two groups. However, the lack of an evidence base has led to variations in practice both between and within countries: the median eGFR at dialysis start is currently 8.2 and 7.8 mL/min/1.73 m^2 for children in Europe and the United States, respectively [4, 14], with a trend towards an earlier start of dialysis reported in the USRDS registry [13].

While formal estimates of GFR are neither practical nor useful in CKD5, KDIGO suggests that risk equations can be helpful in predicting a time frame for when KRT may be needed. In pediatric practice, two formulae have been developed to prognosticate progression to ESKD. Using the Chronic Kidney Disease in Children (CKiD) study cohort, the Kidney Failure Risk Equation uses age, sex, eGFR, and albumin/creatinine ratio (a 4-variable equation) to estimate the rate of progression to dialysis or transplant [15]. A second equation using a multinational collaborative study in 1169 children (ages 1–16 years with an eGFR of 15–90 ml/min/1.73 m^2 and a median follow-up of 3.8 years) is based on eGFR, proteinuria and the CKD diagnosis (glomerular vs. nonglomerular) to determine CKD progression [16].

Dialysis Modality Selection

Dialysis modalities include in-center conventional HD or hemodiafiltration (HDF), home HD, and continuous ambulatory or automated peritoneal dialysis (PD). All extracorporeal dialysis modalities (HD, HDF, and home HD) are together referred to as HD in this chapter. The principles and practice of PD is described at length in ▶ Chap. 75, "Peritoneal Dialysis." There are no robust data to suggest that one modality is superior to another and both HD and PD have relative advantages and disadvantages.

The only absolute contraindication for maintenance HD is the absence of suitable vascular access. All other health conditions are relative contraindications, and therefore, the selection of dialysis modality needs to reflect informed patient choice with decision support appropriate to the health care system. Patients and caregivers need to be informed of the challenges, considerations, and trade-offs of the different dialysis modalities so that modality selection can be tailored to their health and social circumstances [17]. Although preserving residual kidney function is important, it should not be the sole consideration in selecting the initial dialysis modality.

Physiology of Hemodialysis

All dialysis modalities operate under physical principles related to the movement of molecules in aqueous solutions across semi-permeable membranes. Understanding these basic principles allows full recognition of the processes engaged in dialysis therapies and gives the operator more control over the prescription and the procedure. These processes include diffusion, convection, and ultrafiltration. Molecules in the bloodstream will also adsorb to the dialyzer and blood tubing, which represents an additional, though ill-defined and uncontrolled, mechanism of molecular removal.

(i) ***Diffusion*** describes the movement of molecules from an area of higher concentration to an area of lower concentration. In an aqueous system with two chambers separated by a semipermeable membrane (Fig. 1a), dissolved molecules will tend to move by random motion across the membrane down their concentration gradient. Net movement ceases when the particle concentration is equal across both compartments. Smaller molecules diffuse more quickly than larger molecules; this relates to the relative amount of energy required to cause molecular movement. The membrane will restrict movement of any particle that is larger than the membrane pore diameter despite a favorable concentration gradient. Small molecules bound to larger molecules such as proteins behave as if they were larger and may not diffuse; only the free fraction would be able to cross the membrane. Diffusion is bi-directional; molecules may move in either direction until achieving equilibrium. Dialysis therapies that employ a dialysis fluid, such as HD, operate by diffusion. Molecules in the blood will diffuse across the dialyzer membrane into the dialysis fluid, becoming the spent waste dialysate, while those substances within the dialysis fluid that are at higher concentration than the blood have the potential to diffuse across the membrane into the patient.

(ii) C***onvection*** describes molecular movement across a semipermeable membrane under the force of pressure; the net pressure vector pushes water, with dissolved solutes, across the membrane (Fig. 1b). As opposed to diffusion, in which smaller molecules move with greater ease, small or larger molecules will move across the membrane at an equal rate since movement is not dependent on energizing the particles; all solutes up to the cut-off size of the membrane pores pass equally. The net pressure vector arises from a combination of positive pressure on the source side of the system and negative pressure on the destination side, leading to a unidirectional molecular movement.

(iii) ***Ultrafiltration*** describes the movement of the water itself. In hemodialysis, blood flow through the dialyzer generates a positive hydrostatic pressure; the hemodialysis system governs the net pressure vector by adjusting

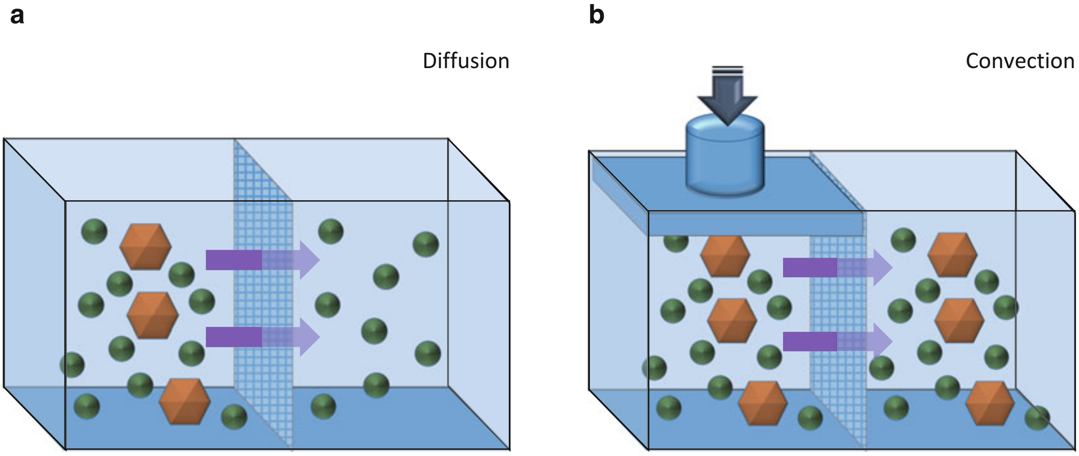


Fig. 1 The principles of diffusion (**a**) and convection (**b**) across a semi-permeable membrane

the hydrostatic transmembrane pressure applied to the dialyzer. Ultrafiltration and convection always occur simultaneously.

Technical Aspects of Hemodialysis

Figure 2 provides a schematic of the hemodialysis system, illustrating the blood and dialysate circuits.

The Blood Circuit

The blood circuit carries the patient's blood to the dialyzer and then returns the blood to the patient via the vascular access. Analogous to arterial and venous systems in relation to the heart, the arterial limb of the blood circuit moves blood away from the patient towards the dialyzer and the venous limb carries blood from the dialyzer to the patient. Monitoring systems within the arterial and venous limbs measure dynamic pressure throughout the dialysis session, providing an assessment of appropriate flow. Abnormal pressures generate alarms that prompt the operator to check for blood flow obstruction or disconnection. The venous limb will also have an air detector to monitor for entrained air within the blood circuit. When triggered, the air detector stops the blood pump and clamps the venous limb to prevent air entry to the patient.

Tubing for the blood circuit must be compatible with the chosen HD device; many companies offer HD systems that require purchase of their proprietary tubing sets while others may permit generic tubing. When prescribing the HD session, one must consider the total extracorporeal blood volume within the tubing set and the hemodialyzer relative to the patient's circulating blood volume. Most manufacturers offer tubing sets in several sizes to

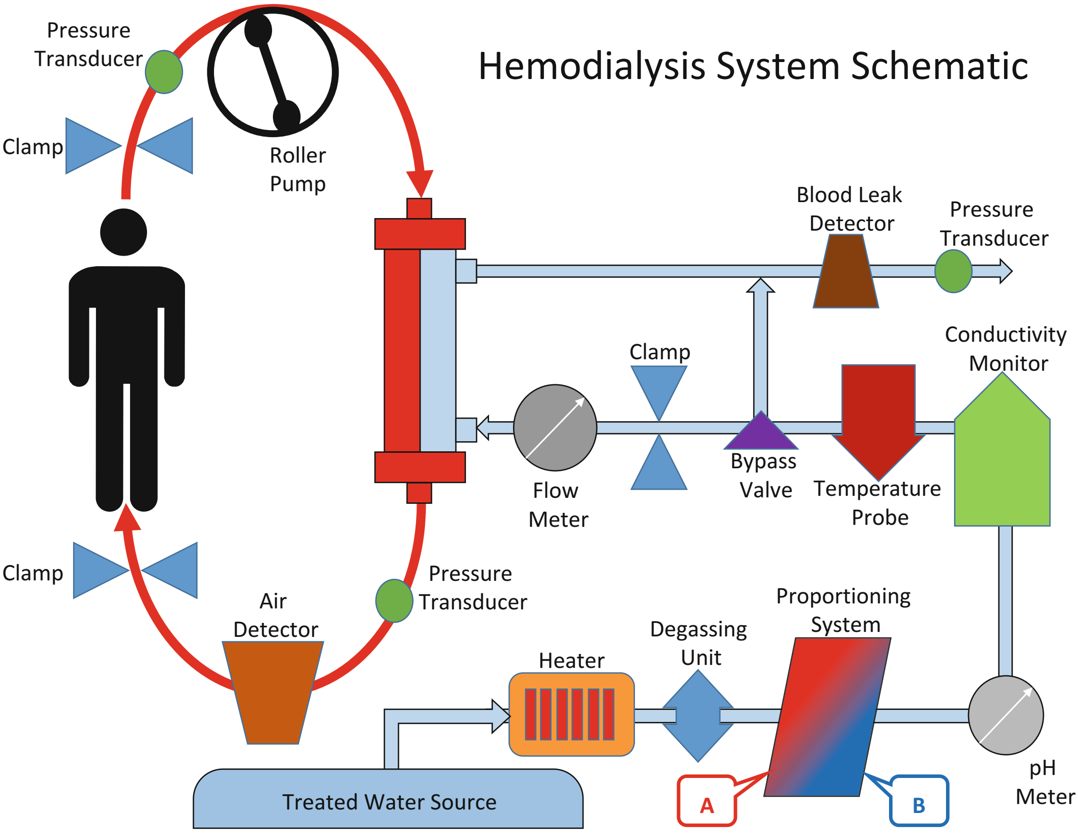

Hemodialysis System Schematic
Pressure Transducer
Clamp
Roller Pump
Blood Leak Detector
Pressure Transducer
Clamp
Conductivity Monitor
Flow Meter
Bypass Valve
Temperature Probe
Clamp
Air Detector
Pressure Transducer
Proportioning System
Degassing Unit
Heater
Treated Water Source
A
B
pH Meter

Fig. 2 A schematic diagram showing the blood and dialysate circuits

accommodate smaller pediatric patients; pediatric hemodialysis teams may need to review their options when considering purchase of HD systems to ensure that they can safely accommodate all patients in their pediatric group.

The HD system draws blood from the vascular access via a rotating roller pump at the arterial limb of the blood circuit. Blood pump rotation generates flow rates ranging from a minimum of 10–30 ml/min to a maximum up to 500 ml/min; this is an important parameter chosen by the operator to prescribe the dose of HD. The roller pump must maintain appropriate occlusive pressure on the portion of the tubing within the pump housing device to produce consistent forward flow and to avoid complications including hemolysis or foaming of blood within the circuit. Pressure variances and inappropriate flow may lead to audible “thump” or “knock” with each revolution of the blood pump or trigger an alarm that stops the blood pump for safety.

Hemodialyzers

Modern hemodialyzers are of hollow-fiber design comprised of multiple capillary tubes manufactured from semipermeable material through which blood flows, embedded within a cylindrical plastic cartridge in which dialysate bathes the exterior of the artificial capillaries (Fig. 3). Each capillary tube represents a semipermeable membrane across which diffusion, convection, and ultrafiltration may occur. Early HD membranes were made of cellulosic material but had a risk of causing allergic reactions. As a result, hemodialyzer composition evolved through modified cellulosic materials to fully artificial (plastic) biocompatible membranes. Manufacturers develop and market numerous variations of dialyzer membranes, which may differ in porosity, thickness, surface charge, and relative biocompatibility. Clinicians who prescribe hemodialysis should be knowledgeable regarding these differences to make appropriate choices for patients and avoid complications [18].

Hollow fiber dialyzers come in various sizes to permit safe and effective HD for patients of all ages and sizes (Table 1). Smaller dialyzers, appropriate for younger children, will have a lower priming volume, the amount of blood necessary to fill the dialyzer during the session. Larger dialyzers will have larger priming volumes. Similarly, the surface area available for dialysis will vary with the size of the dialyzer; smaller dialyzers will have lower surface areas compared to larger dialyzers. These factors prove important when developing the prescription for a patient as membrane surface area has an impact on HD efficiency and priming volume of the dialyzer is a component of total extracorporeal blood volume.

All dialyzers undergo sterilization by the manufacturer before sale. Some sterilizing methods

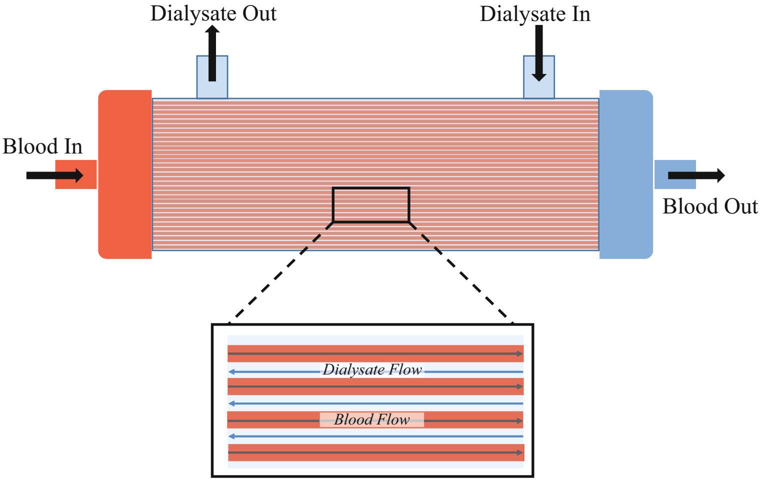


Fig. 3 Hemodialyzer schematic showing counter-current flow of blood and dialysate

Table 1 Dialyzer characteristics, options, and considerations for pediatric hemodialysis

Dialyzer characteristic	Range of options	Considerations
Surface area	0.2 m^2–2.5 m^2	Larger surface gives greater capacity for clearance and ultrafiltration but requires high blood flow to achieve these advantages
Priming volume	18 ml–140 ml	Consider along with tubing set volume to determine maximum safe extracorporeal volume, especially for smaller patients
Membrane material	Cellulosic, modified cellulose, artificial	Cellulosic membranes considered less bio-compatible and may lead to more allergic-type reactions; greater bio-compatibility with progression to modified cellulose and then to artificial (plastic) membranes
Sterilization	Ethylene oxide (ETO), gamma irradiation, steam, electron beam	Allergic reactions associated with ETO; fewer reactions with gamma and steam
Flux	Low flux vs. high flux	Higher flux dialyzers have higher permeability (e.g., larger pores), permitting higher rates of middle molecule clearance and higher UF rate at a given prescription
Clearance (typically described as K_D for urea (ml/min) at Q_B 200 ml/min)	K_0A urea 170 ml/min – 1700 ml/min	Varies with surface area, flux, membrane material
Ultrafiltration capability (K_{UF} ml/h/mmHg)	K_{UF} 7 ml/h/mmHg – 111 ml/h/mmHg	Varies with surface area, flux, membrane material

may leave residual chemicals within the dialyzer that require rinsing and may put the patient at risk for reactions at the time of the procedure. This has been well described with ethylene oxide (ETO), a formerly ubiquitous method for dialyzer sterilization. Many manufacturers now sterilize by steam or gamma irradiation (Table 1) to avoid this concern.

Within the HD circuit, the dialyzer serves as the "artificial kidney." Two dialyzer characteristics are important for treatment effectiveness: the porosity of the membrane and the surface area available for diffusion. These together define the permeability surface area product (K_0A). The K_0A, expressed in ml/min, describes the maximum diffusion capacity of a dialyzer for a given solute. A larger dialyzer with greater surface area will have a higher K_0A than would a smaller dialyzer made from the same membrane material. Two dialyzers of identical size and surface area may not share the same K_0A due to differences in membrane material and relative permeability. The clearance (K_D) of a dialyzer, also with units of ml/min, describes the ability of the dialyzer to remove a given molecule from the blood in a unit time under the conditions of a specific dialysis prescription. K_D is a function of the K_0A but also varies with the blood flow rate (Q_B) and dialysis fluid flow rate (Q_D).

The ultrafiltration coefficient (K_{UF}) is an analogous concept. Expressed with units of ml/h/mmHg, the K_{UF} describes the ability of the dialyzer to ultrafilter a volume of water per unit of time at a given transmembrane pressure. Dialyzers with higher K_{UF} will efficiently ultrafilter at lower transmembrane pressures; a dialyzer with a lower K_{UF} will not be able to increase ultrafiltration above its limit despite the application of higher transmembrane pressures. One must consider K_0A, K_D, and K_{UF} when developing the dialysis prescription for an individual patient (see below).

The Dialysis Fluid Circuit

The dialysis fluid is the aqueous solution into which substances from the blood will diffuse.

The dialysis fluid circuit includes components to generate and test the fluid before it enters the dialyzer and a system to deliver the dialysate to the dialyzer. Historically, it was necessary to prepare the total volume of dialysate for HD sessions ahead of time. On-line preparation of dialysis fluid, using a proportioning system to combine dialysis-grade water and electrolyte concentrates to generate just-in-time dialysis fluid during the HD session, largely has replaced the original "batch" systems [19].

To ensure safety, one must minimize the presence of molecules within the dialysis fluid that may pose a risk to the patient. Municipal water may have high mineral content, particulate matter, or additives such as chlorine, which may make the water acceptable for ingestion but can harm a dialysis patient should they diffuse into the bloodstream. The water treatment system, therefore, is an essential component of any HD unit [20]. Combining particle filtration, water softeners, activated charcoal perfusion and reverse osmosis membranes, current water treatment systems make tap water suitable for dialysis. Governmental agencies around the world list specific guidelines for dialysis water, specifying expected levels of purity, and monitoring regimens to which dialysis units must adhere.

The concentration of electrolytes in dialysis fluid should approximate those of serum, with adjustments to correct the imbalances related to kidney failure. Concentrates of electrolytes, available as liquids or bulk powders, when mixed with the water, make dialysis fluid. For bicarbonate-based dialysis fluid, separation of dialysis fluid concentrates into two components prevents precipitation and simplifies storage. The acid component, often denoted as "A" and color-coded red, contains sodium, calcium, magnesium, potassium, glucose, and chloride, combined with an organic acid. One may choose from a variety of "A" component formulations to yield a final dialysis fluid with the desired electrolyte content; in particular, there are options for potassium and calcium concentration depending on the clinical situation. The base component, marked as "B" and color-coded blue, contains the bicarbonate.

The proportioning system within the dialysis machine draws up "A" component, "B" component, and water to blend the three streams into dialysis fluid. The system tests the dialysis fluid by continuously measuring its conductivity to assure the electrolyte content is accurate before delivery to the dialyzer. If conductivity falls outside of the expected range, the system diverts the dialysis fluid away from the dialyzer and the machine alarms to notify the operator. The mixed dialysis fluid enters the dialyzer cartridge and bathes the blood-filled capillary fibers, providing a destination for diffusion. To optimize diffusion efficiency, blood and dialysis fluid flow in opposite directions within the dialyzer (counter-current flow). Settings for dialysis fluid flow rate may be limited depending on the brand of dialysis machine; common options are a minimum rate of 300 ml/min and maximum rate of 800 ml/min.

Ultrafiltration Control

Earlier generations of dialysis machines required manual calculation and adjustment of the transmembrane pressure to achieve ultrafiltration (UF) goals. This process was fraught with risk to the patient due to inaccurate UF. Modern dialysis machines include automated UF control systems that precisely monitor transmembrane pressure and the rate of water transit across the dialyzer to assure accuracy of fluid removal. Every dialysis system has an inherent level of UF inaccuracy which is quantified in the dialysis system specifications. As a result, the prescribed UF can differ from the actual UF, necessitating careful monitoring and assessment of the patient's volume status throughout the dialysis treatment, especially for smaller children.

Vascular Access

A well-functioning vascular access is crucial to the success of dialysis. There are three types of vascular access for chronic HD: central venous lines (CVLs) which are tunneled cuffed catheters placed in a central vein, arteriovenous fistulas

(AVFs), and arteriovenous grafts (AVGs) using prosthetic or biological material. The ideal vascular access is one that provides adequate blood flow for solute clearance and has a low risk of infection or causing damage to the vasculature. There is robust evidence to recommend AVFs over CVLs in adult patients, and pediatric studies suggest similar outcomes with improved dialysis clearance, fewer infections, fewer hospitalizations, and fewer access changes [21–24]. Guidelines on vascular access for chronic HD recommend the use of AVFs over CVLs where feasible [25, 26]. However, AVFs may not be suitable in the very small child or those with an anticipated short dialysis course before transplantation or transition to PD, so the Fistula First program [21] may not be appropriate for all children.

Prevalence of Different Access Types Used Globally

Although AVFs are recommended as the preferred vascular access for the majority of children, CVLs remain the most frequently used access type in all pediatric age groups [14, 26–28], possibly because of the perceived convenience of CVLs. The International Pediatric Hemodialysis Network (IPHN) reported that in over 550 children from 27 countries only 26% of all access types were AVFs, and CVLs were the predominant access type in 72% [27]; this trend was also noted in children older than 10 years. Data from the European Pediatric Dialysis Working Group demonstrate a different proportion with 38% of all access types being AVFs [29]. Across all registries, AVGs are used in <2% of children and usually as a last resort when other options have been exhausted [27, 29]. Recent guidelines from the European Society of Pediatric Nephrology Dialysis Working Group (EPDWG) suggest that children requiring chronic HD should start with a functioning AVF where appropriate but recognize that not every child is a suitable candidate for AVF formation [26]. A dedicated vascular access clinic that includes dialysis nurses, play therapists, and psychologists [30] is a useful “hub” to promote patient education [23, 31] regarding preferential access options.

CVL Types and Sites of Placement

One must consider and carefully choose the type and size of catheter, catheter site, and technique for insertion for each patient. The internal jugular vein is the preferred site. CVLs in the femoral vein are associated with a higher risk of infection and thrombosis [32] so should be avoided except in emergencies or where all other access has failed. Subclavian catheters are associated with a high risk of postoperative stenosis [33], limiting options for future vascular access. Although there are no comparative studies in children, a fluoroscopic or ultrasound-guided technique for CVL insertion may be considered to prevent mechanical complications at the time of CVL insertion. The IPHN report suggests that vascular access dysfunction was more common when the CVL was placed in the femoral (56%) or subclavian vein (35%) as compared to the internal jugular vein (21%) [27].

AVF Formation

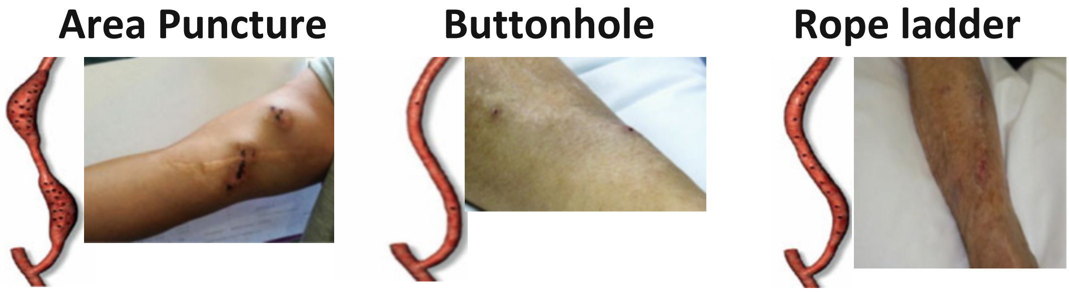
An AVF is created by surgically connecting an artery to a vein, which transmits higher pressure to the vein, causing it to expand, after which large enough needles may be inserted to provide the high blood flows required for dialysis [34]. The AVF may be created at the wrist (radiocephalic) or the elbow (brachiocephalic) or by basilic vein transposition to create a brachiobasilic fistula. It is preferable to create the first AVF distally at the wrist to preserve more proximal vessels for future use. The nondominant arm is preferred for AVF formation as this will allow for self-needling of the AVF, easy use of the dominant arm during dialysis, and limit any potential neurovascular complications of surgery.

While some groups are able to create AVFs in very young children using microsurgery [34], larger vessels are usually associated with higher rates of AVF maturation and successful use. Vein mapping prior to AVF surgery can direct the surgery to an anatomical site that has the best chance of success. In general, the intraluminal diameter of the vein should be at least 3 mm. In small patients, a two-stage basilic vein transposition may be preferable; the anastomosis is created in the first stage and about 4–6 weeks later the arterialized vein is brought to the surface to make access easier [34]. There are no randomized studies comparing different sites of AVF formation in adults or children but registry data suggests that radio-cephalic AVFs have excellent primary and secondary patency rates of 78–85% and 94%, respectively, comparable to those for brachiocephalic AVFs [35]. The success of AVF depends on adequate outflow distal to the anastomosis, or else high venous pressures may result. Thus, a child who has had previous central lines may need upper limb venography to confirm patency of the central vessels [36]. Ultrasound examination of the proximal vessels can be misleading and should not be relied upon because collaterals may be mistaken for patent upper limb vessels, and veins cannot easily be seen under the clavicle.

AVF Cannulation

Two prospective studies in children have reported an average maturation time of 8–10 weeks for the majority of AVFs [31, 37] with no significant difference between AVFs at different anatomical sites [27]. One study suggests that the timing of first AVF needling should be delayed to at least 45 days after AVF creation, as this has been associated with improved primary and secondary patency rates compared to earlier AVF cannulation [38]. The first AVF cannulation usually starts with a single needle of a smaller size, and in some centers with ultrasound guidance, gradually building up to larger needle sizes and two needles.

An AVF can be cannulated by the rope ladder, buttonhole, or area puncture technique (Fig. 4). While the expertise and preference of the person cannulating an AVF is a critical factor, systematic reviews of trials in adult HD patients do not show



Area Puncture

Technique:
- Single cannulation site in one small area
- Both cannulation sites on the same segment but do not meet
- Sharp needles

Benefits:
- Patient choice – needle phobia
- Small AVF – space
- Reduced infiltrations

Disadvantage
- Bleeding, aneurysms, AVF thrombus
- Stenosis
- Body image

Buttonhole

Technique:
- Same hole in the skin, same place in the vein; same person needling to establish a track
- Pick scabs; start with sharp needles
- Blunt needles once track has been established
- 3 – 4 buttonholes along vessel

Benefits:
- Less pain with needle insertion
- Reduced bleeding time post needle removal
- Fewer missed cannulations
- Decreased risk of aneurysm formation
- Promotes self cannulation

Disadvantage:
- Scab picking; increased infection risk
- Easy to mistake for area puncture

Rope ladder

Technique:
- Over at least 8cm segment
- Each site 0.5-1cm above previous
- Sharp needles
- Cannulation -zip / central or traditional / side to side, moving up the vein

Benefits:
- Reduced risk of stenosis or aneurysm formation
- Lower infection risk

Disadvantage:
- Needs long segment of fistula vein
- Harder needle insertion
- Increased risk of infiltration
- Still requires planning

Fig. 4 A schematic diagram showing the different techniques of arteriovenous fistula cannulation, and the relative advantages and disadvantages of each technique

a clear benefit of buttonhole cannulation in terms of patient survival, access survival, quality of life, needling pain, infection, bleeding during or after dialysis, and aneurysm formation [39, 40]. A recent cross-sectional survey of cannulation practices suggests that the area puncture technique was associated with a higher rate of access failure than the rope ladder or buttonhole techniques, and is no longer recommended [41]. Buttonhole cannulation carries a higher risk of access infection [42] and must be avoided in children with frequent skin infections.

Outcomes of AVFs and CVLs

The IPHN data suggest that AVFs have better event free survival probability and higher primary and secondary patency rates up to four years after creation compared to CVLs [27]. Having a CVL increased the need for new access at a different site by two-fold [27]. In addition, multiple retrospective studies in children have shown that CVLs present a significantly higher infection rate, risk of access related sepsis, and higher rates of hospitalization than AVFs [22, 23, 28, 37, 43].

Prescribing Hemodialysis and Special Considerations for Children

HD prescription for children requires careful review and a patient-specific approach. Standardized prescriptions may lead to inappropriate doses of dialysis, putting the patient at risk for complications. The following section of this chapter describes the components of a pediatric dialysis prescription and methods for its development; prescription and monitoring of dialysis dose is described in ▶ Chap. 78, "Adequacy of Dialysis."

Blood flow rate through the HD circuit (Q_B) is a major determinant of dialysis solute clearance; the vascular access determines the maximum Q_B that is achievable. Since the resistance to flow through a tube (e.g., blood through a blood vessel or a CVL) is inversely proportional to the fourth power of the radius, larger central venous catheters and AVFs will tend to allow higher Q_B and permit more efficient hemodialysis. Conversely, smaller vascular access (e.g., in infants and younger children) limits the maximum Q_B achievable for treatment. To prevent saturation of the dialysate and consequent reduction of diffusion capacity, the dialysis fluid flow rate (Q_D) is required to be 1.5–2 times that of Q_B.

When selecting a dialyzer for chronic HD, consider the dialysis goals for the session and the volume of the dialyzer. The dialyzer forms part of the extracorporeal circuit volume; perfusing a HD circuit with a blood volume more than 10% of the patient's intravascular volume increases the risk for hypotension and shock. As a general rule, the dialyzer membrane surface area should be approximately equal to the patient's body surface area. Practitioners should also consider the dialyzer membrane material and sterilization method, given risks for reactions during the session.

With the choice of Q_B, Q_D, and dialyzer, one may then determine the predicted clearance (K_D) for the session. Since urea is a small molecule that distributes in the total body water and is easy to measure in the patient's blood, it is a convenient marker for small molecule clearance during dialysis. Dialyzer manufacturers publish urea clearance (K_D(urea)) values for their dialyzers but may only report those clearances associated with higher Q_B as performed for adults; if a child's vascular access cannot generate Q_B within the listed range, K_D(urea) must be estimated and is usually approximates the Q_B itself.

With a value for K_D(urea) determined, all necessary data are present to calculate the time for dialysis using the mass transfer equation. The mass transfer equation describes the diffusion of a given molecule from the blood into the dialysis fluid. The mass transfer equation has the following format:

$$C_t/C_0 = e^{-Kt/V}$$

The term "e" is the base of the natural logarithm function. The variables in the mass transfer equation are:

C_t = the concentration in the blood of the molecule in question at time "t."
C_0 = the concentration in the blood of the molecule in question at time zero (start of HD).
K = the dialytic clearance of the molecule in question.
t = the time of the dialysis procedure in minutes,
V = the volume of distribution of the molecule in question in milliliters.

If the molecule in question is urea, one may use the K_D(urea) and the other known variables to solve the equation for the length of the dialysis session (t) to achieve the desired urea clearance. The mass transfer equation permits one to model the change in blood concentration of a substance under the effects of the dialysis procedure. This allows control over the prescription and the ability to achieve the goals for a HD session.

Consider the following clinical example using the mass transfer equation to determine a first prescription for HD:

> A patient weighing 50 kg with previously undiscovered kidney disease presents with a blood urea nitrogen (BUN) concentration of 100 mg/dl. The patient will require HD; the first HD session often targets lower urea reduction to prevent the complications of dialysis dysequilibrium (see below). A 30% reduction in BUN (goal 70 mg/dl) would be reasonable. The acutely placed CVL is likely to deliver Q_B of 200 ml/min; data table for the selected dialyzer reports K_D(urea) of 185 ml/min at Q_B 200 ml/min. Total body water can be estimated as 60% of the body weight (30 kg or 30,000 ml). Using these data, re-arrange the mass transfer equation to solve for time of the HD session (t):
>
> $t\ (\text{minutes}) = -\ln (C_t/C_0) \times (V/K)$
> $t\ (\text{minutes}) = -\ln (70/100) \times (30{,}000/185)$
> $t\ (\text{minutes}) = -\ln (0.7) \times 162.2$
> $t\ (\text{minutes}) = -(-0.357) \times 162.2$
> $t\ (\text{minutes}) = 0.357 \times 162.2$
> $t\ (\text{minutes}) = 58$

While many patients will dialyze using a standard dialysis fluid, some patients require adjustment to the more commonly used blend (Table 2). Choosing different options for the "A" component can yield varied concentrations for potassium in the final dialysis fluid. A common default concentration for potassium in the dialysis fluid is 2 mmol/L, chosen to generate an outward diffusion gradient and negative potassium balance for a patient who lacks kidney function. The potassium bath concentration choice requires caution and careful thought; an overly rapid decrease of potassium when dialyzing against a very low concentration may cause cardiac arrhythmias. For this reason, the use of dialysis fluids without any potassium (zero K baths) should be strictly prohibited. For individuals who undergo dialysis daily and have poor dietary intake, dialyzing against a 2 mmol/L potassium bath may lead to total-body potassium depletion; such a patient may benefit from dialyzing with a 3 mmol/L potassium bath. One may take a similar approach when choosing from different options for the calcium concentration, with normal values between 1.25 and 1.75 mmol/L (2.5–3.5 meq/L).

Table 2 Common dialysate composition for hemodialysis

Component	Dialysis fluid concentration	
	US units	SI units
Sodium	137–144 mEq/L	137–144 mmol/L
Potassium	0–4 mEq/L	0–4 mmol/L
Chloride	98–112 mEq/L	98–112 mmol/L
Bicarbonate	27–40 mEq/L	27–40 mmol/L
Calcium	5–7 mEq/L	1.25–1.75 mmol/L
Magnesium	0.61–2.43 mEq/L	0.25–1 mmol/L
Glucose	0–198 mg/dl	0–11

One may adjust other components of the dialysis fluid concentration including the sodium and bicarbonate levels. However, the method involves manipulating the blend of "A" component, "B" component and water in the proportioning system. Consequently, these adjustments lead to changes in the concentrations of all components of the dialysis fluid.

Consider the following clinical example to illustrate choice of dialysate:

> The patient in the example above, presenting for first dialysis session with previously undiscovered kidney failure, has other biochemical abnormalities in addition to initial BUN of 100 mg/dL. Blood testing prior to first dialysis session includes the following results:

Patient values	US units	SI units
Sodium	137 meq/L	137 mmol/L
Potassium	5.8 meq/L	5.8 mmol/L
Chloride	102 meq/L	102 mmol/L
Total CO2	16 meq/L	16 mmol/L
Calcium (total)	6.8 mg/dL	1.7 mmol/L
Phosphorus	10.1 mg/dL	3.3 mmol/L
Albumin	2.9 g/dL	29 g/L

Of note, the patient presents with above-normal potassium, low total CO2 indicative of metabolic acidosis, low calcium, and high phosphorus. A reasonable choice for dialysis fluid concentrations may be as follows:

Dialysis fluid	US units	SI units
Sodium	140 meq/L	140 mmol/L
Potassium	2 meq/L	2 mmol/L
Bicarbonate	30 meq/L	30 mmol/L
Calcium (total)	3.5 meq/L	1.75 mmol/L

Note that the potassium concentration of the dialysis fluid is lower than that of the patient, permitting diffusion and removal, but the gradient is not so large as to potentially increase the risk for arrhythmia. The bicarbonate concentration is higher than that of the patient, which will permit some correction of the metabolic acidosis, but is somewhat lower than that used in more routine sessions to avoid an overly rapid correction which could exacerbate risk for arrhythmia due to intracellular potassium shift. The patient's hypocalcemia prompts use of a higher dialysis fluid calcium concentration to support correction and again reduce risk of arrhythmia. Daily HD sessions for 3–5 consecutive days may be needed to correct the imbalances slowly and safely, after which one may consider transition to a more routine dialysis fluid concentration such as the following:

Dialysate fluid	US units	SI units
Sodium	140 meq/L	140 mmol/L
Potassium	2 meq/L	2 mmol/L
Bicarbonate	35 meq/L	35 mmol/L
Calcium (total)	2.5 meq/L	1.25 mmol/L

Note that the potassium concentration in the dialysis fluid is most often 2 meq/L (2 mmol/L) for thrice-weekly HD to address potassium accumulation in the interdialytic period. Adjustments to the dialysis fluid concentration may be required for patients on different HD schedules, as one may see with home HD (see below).

The acidifying agent in the "A" component is most commonly acetic acid. Citric acid may serve as an alternative [44, 45]. If used, the citrate in the dialysis fluid may provide some local anticoagulation at the membrane, improving dialyzer efficiency. Some users report reduction of or complete elimination of the need for heparin anticoagulation during dialysis sessions using citrate based dialysis fluid [46].

The dialysis machine controls the dialysis fluid temperature, which can vary 1–2 degrees Celsius around the standard temperature of 37 °C. Colder dialysis fluid may limit hypotension and hypoxia during the dialysis session [47–49] but too low a temperature causes patients to feel cold, which they tolerate poorly.

Many patients with renal dysfunction undergoing HD will have volume excess due to oliguria. During the HD session, one must choose a goal volume of fluid to remove from the patient via UF. Modern automated UF control systems permit the operator to program a goal fluid target and for the dialysis machine to govern the transmembrane pressure, yielding the target volume of ultrafiltrate in the prescribed time. Clinical factors related to the patient and technical aspects of the therapy deserve consideration when planning the UF goals, as outlined next.

While excess fluid within the patient may distribute to multiple body compartments, the HD procedure only has access to the vascular compartment. Even in an otherwise healthy patient without systemic illness or cardiac dysfunction, overly rapid removal of fluid from the vascular compartment by UF may lead to symptomatic hypotension while not removing the excess fluid that remains within the interstitial space. One may mitigate the risk for hypotension through slower rate of UF. This may lead to insufficient fluid removal in the prescribed session time or may necessitate extending the length of the procedure to achieve the goal with a slower UF rate. It is easier to achieve UF goals for patients who have a smaller volume of fluid to remove and who have normal vascular tone; UF is more difficult to manage for patients who have significant volume overload (an interdialytic weight gain of >8%) or have poor vascular status, as may be seen in critically ill patients with sepsis. Thus, those undergoing HD in the setting of systemic diseases including sepsis or multiorgan dysfunction may benefit from either daily HD sessions with lower per-session UF targets to maintain fluid balance or, if available, extended or continuous forms of kidney replacement therapy that will distribute the UF goal throughout the day (see ▶ Chap. 67, "Evaluation and Management of Acute Kidney Injury in Children"). Those patients with cardiac dysfunction are also at increased risk for complications with rapid ultrafiltration as they may not be able to compensate for intravascular volume removal with increased cardiac output, and their risk for myocardial hypoperfusion will be increased (see below). Younger children may be particularly prone to symptomatic hypotension due to their relatively small intravascular volume; careful bedside monitoring by experienced dialysis staff, sometimes with the assistance of technology such as noninvasive blood volume monitors, can help to assure safer and more comfortable HD sessions while achieving appropriate ultrafiltration goals [50, 51]. For maintenance HD sessions, many programs will choose to limit, by policy, maximum UF goal per session (e.g., no greater than 5–8% of the patient's current target "dry" weight) or UF rate (e.g., no greater than 10–13 ml/kg/h) to remain in a "safe zone" that allows appropriate compensation between intravascular and extravascular fluid compartments. As noted above, this is easier to achieve with limited intradialytic fluid gain, longer HD sessions, or daily HD schedules; in conventional thrice-weekly HD, it may be challenging to remain within these parameters and also achieve appropriate volume control [52].

Anticoagulation for Hemodialysis

Membrane and tubing surfaces in the blood circuit activate the coagulation cascade and cause clotting. Most dialysis circuits will require anticoagulation to perform HD successfully. Systemic unfractionated heparin has been the longstanding anticoagulant for HD. Most circuits will receive an initial bolus of heparin at the start of the HD procedure, followed by a continuous infusion at a lower rate to maintain anticoagulation throughout the session. Alternatives methods include bolus at session start without infusion or infusion only without starting bolus; variations may depend on local practice or on patient-specific issues. While effective, heparin has the disadvantage of being a systemic anticoagulant that may predispose the patient to bleeding; this is less commonly a problem with intermittent HD sessions since the exposure time is four hours or less. Heparin-induced thrombocytopenia is possible but relatively uncommon in the pediatric population; when it occurs, one must suspend heparin use. Alternatives to systemic heparin include regional citrate anticoagulation or use of citrate-containing dialysate which provides local anticoagulation at the dialyzer membrane [45, 46]. If anticoagulant-free dialysis is required, intermittent flushing of the dialyzer with saline may be performed, but this may lead to a high sodium and volume load to the patient.

Hemodiafiltration

Standard HD clears uremic toxins primarily through diffusion, with clearance being inversely proportional to the molecular size, protein binding, and tissue distribution of the toxin. Recognizing the importance of larger uremic toxins, it is evident that there is a need for alternative therapies that provide better removal of those solutes, leading to the development of a combined convective and diffusive therapy, hemodiafiltration (HDF). Figure 5 shows a schematic presentation of HD and HDF circuits.

Definition of HDF

The European Dialysis (EUDIAL) working group has defined HDF as a blood purification therapy that combines diffusive and convective solute removal by ultrafiltration of 20% or more of the blood volume processed through a high-flux dialyzer and maintenance of fluid balance by sterile replacement fluid infused directly into the patient's blood [53]. In on-line HDF, large volumes of sterile replacement fluid are obtained by

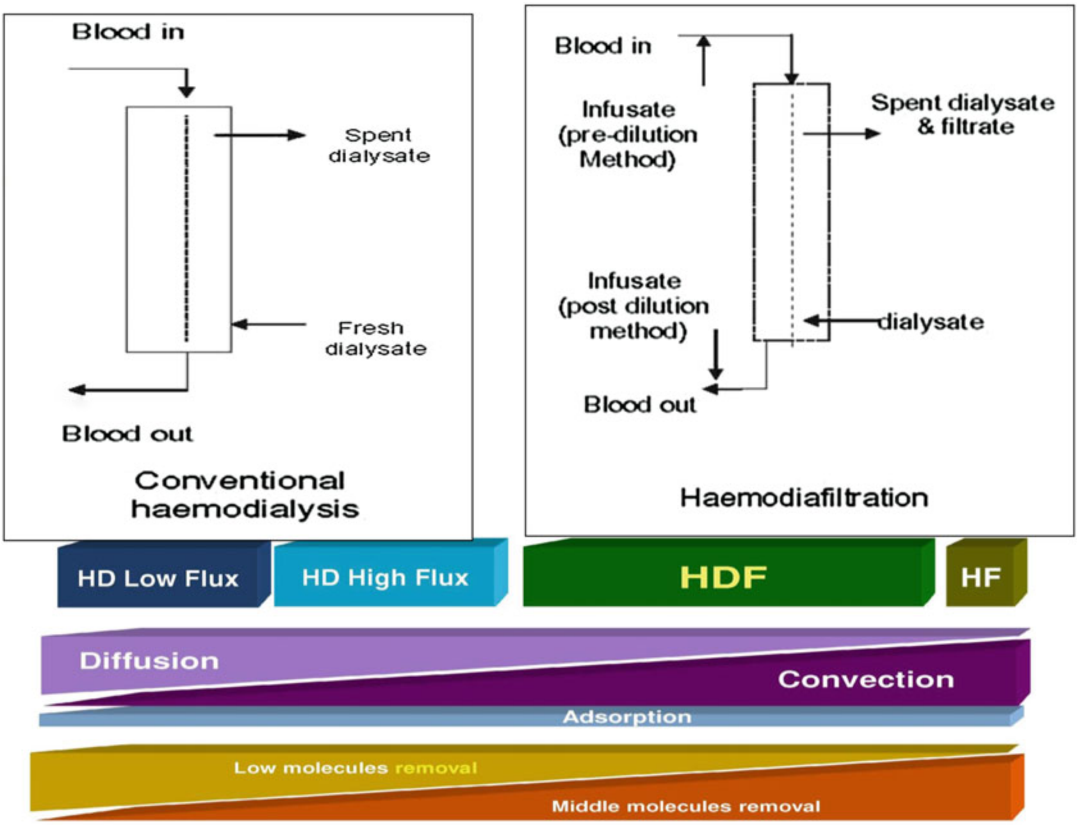

Blood in
Spent dialysate
Fresh dialysate
Blood out
Conventional haemodialysis
Blood in
Infusate (pre-dilution Method)
Spent dialysate & filtrate
Infusate (post dilution method)
dialysate
Blood out
Haemodiafiltration
HD Low Flux
HD High Flux
HDF
HF
Diffusion
Convection
Adsorption
Low molecules removal
Middle molecules removal

Fig. 5 A schematic presentation of hemodialysis (HD) and hemodiafiltration (HDF) circuits and their mechanisms of clearance by diffusion alone (HD) vs a combination of diffusion and convection (HDF)

on-line filtration of standard dialysate though a series of bacteria- and endotoxin-retaining filters [54]. A high convective volume is a fundamental requirement for HDF. The convective volume is the sum of the net UF volume (i.e., the amount of fluid removed during a dialysis session based on the interdialytic weight gain) and the amount of substitution fluid (i.e., the sterile replacement fluid given as replenishment for the removal of extra fluid during HDF).

Convective clearance optimizes the removal of middle (up to 300–500 Dalton [Da] molecular weight) and larger (greater than 15–50 kDa) molecules. Randomized controlled trials [55–58] suggest that any improved survival associated with HDF occurs when the convective volume exceeds 20 liters/session. Modern HDF machines can achieve convective volumes of 30–35% and should be aimed for in order to obtain optimal clearance.

Modes of HDF

The location within the dialysis circuit where one infuses replacement fluid defines the different modalities of HDF (Fig. 5):

(i) ***Postdilution HDF*** **–** The replacement fluid is infused downstream of the dialyzer, usually into the venous bubble-trap. A potential disadvantage is that hemoconcentration at high UF rates can result in the deposition of plasma proteins on the membrane surface, clogging the membrane pores, and occluding the blood channels of the dialyzer. These effects can raise transmembrane pressure (TMP), causing alarms, reducing clearance, and possibly resulting in clotting of the extracorporeal circuit [53].

Hemoconcentration generally limits the convective volume achieved to 20–25% of blood flow rate in postdilution HDF. The ultrafiltration rate is controlled in proportion to the actual blood flow rate or guided by TMP. A filtration fraction up to 35% of the blood flow rate is possible using systems designed to optimize filtration rate, based on automatic adjustment of TMP according to UF flow rate measurements [59].

(ii) ***Predilution HDF*** **–** The replacement fluid is infused upstream of the dialyzer. Ultrafiltration rates up to 100% of blood flow rate are used. Predilution reduces the efficiency of both the diffusive and convective components of solute removal by reducing solute concentrations in the blood compartment, and for equivalent clearance, the convective volume needs to be 2 to 3 times greater for predilution compared to postdilution HDF [60].

(iii) ***Mid-dilution HDF*** **–** The replacement fluid is infused part-way down the blood pathway using specially designed dialyzers – the first part of the blood circuit is operated in post-dilution mode and the second part in pre-dilution mode [61]. Very large filter sizes, up to 1.9 $\mathbf{m^2}$ are required, and hence, this technique is not suitable for children.

(iv) ***Mixed dilution HDF*** **–** The replacement fluid is infused both upstream and downstream of the dialyzer in varying ratios. As with mid-dilution HDF, the need for large filter sizes precludes its use in children.

Postdilution is the most efficient mode of HDF for clearing middle and large molecular weight substances and is the most commonly used HDF technique in adults and children, but in patients with low blood-flow rates, predilution HDF may be preferred.

Requirements for HDF

Essential requirements for performing HDF include:

(i) ***"Ultrapure" water for replacement of convective volume.*** The sterile, nonpyrogenic fluid used to maintain fluid balance, referred to as replacement fluid or substitution fluid, is generated on-line by filtering dialysis fluid through bacteria- and endotoxin-retentive filters to prepare a sterile and pyrogen-free solution that is immediately infused into the patient (therefore also called on-line HDF). The International Organization for Standardization (ISO) has defined ultrapure dialysate as containing <0.1 colony-forming unit/ml and < 0.03 endotoxin unit/ml. Bacteria- and endotoxin-retentive filters installed on the inlet dialysis fluid circuit are key components of the on-line HDF safety system. As large volumes of fluid are removed from, and added to, blood during HDF, strict safety standards and regulatory oversight are required.

(ii) ***High flux membranes.*** Only highly permeable membranes, defined as membranes with an ultrafiltration coefficient (K_{UF}) greater than 20 mL/h/mmHg transmembrane pressure and a sieving coefficient (S) for β_2-microglobulin (β2-m) of greater than 0.6 [53, 62], are suitable for HDF. The K_{UF} defines the hydraulic permeability of a membrane and a high-flux membrane allows a larger and predefined convective flow as required for HDF. As with conventional HD, the dialyzer surface area must be equal to (or slightly higher) than the body surface area, keeping in mind that the internal volume of the dialyzer and blood lines is less than 10ml/kg body weight. For HDF, a biocompatible dialyzer must be selected; biocompatibility is assessed by complement activation, thrombogenicity, contact activation, and cytokine generation [63]. European recommendations state that ultra-pure dialysate must be used with synthetic high-flux membranes [53], both in HDF and HD modalities.

(iii) ***Dialysis machines with accurate ultrafiltration control.*** Many of the new dialysis machines allow for both HD and HDF and are suitable for children above 10–17 kg body weight depending on the machine specifications.

The Concept of Back-Filtration

Since blood and dialysis fluid pass through the filter in counter-current directions, and the hydrostatic pressure of both fluids decreases as they pass through the filter, the resulting TMP may become negative at the venous side, causing an influx of dialysis fluid into the blood compartment of the dialyzer; this is called back-filtration. This phenomenon is a routine occurrence during high-flux HD [64], but not in low-flux HD. The high internal UF rate may increase convective transport of middle molecules [65]. In adults the convective volume achieved by back-filtration is no more than 1–10 L per session depending on the dialyzer type and can vary throughout the dialysis session depending on TMP. Since the convective volume achieved by back-filtration is low and unreliable, it should not be considered a form of HDF. Importantly, given the phenomenon of back-filtration, it has been suggested that dialysis fluid used for high-flux HD should also be sterile and pyrogen-free.

Potential Advantages of HDF over Conventional HD

HDF is thought to be superior to conventional HD through the following mechanisms:

(i) **Improved clearance of toxins across a wide molecular weight range**. HDF clears 70–80% of β2M compared to HD [66] and increase removal of inflammatory cytokines with reduction in inflammation and oxidative stress [67].

(ii) **Improved hemodynamic stability.** HDF permits increased net UF and improves intradialytic hemodynamic stability [68], leading to less intradialytic hypotension [69], reduced incidence of strokes [56], and faster recovery time postdialysis [70].

(iii) **Biocompatibility and reduced inflammation.** The use of "ultrapure" dialysate and increased removal of inflammatory cytokines reduces inflammation and oxidative stress [67].

HDF Studies in Adults

In adults on dialysis, the Estudio de Supervivencia de Hemodiafiltración On-line (ESHOL), one of the largest RCTs comparing HDF vs high-flux HD in adults, has shown that patients on high volume HDF have a survival benefit compared to those on high-flux HD [56]. Pooled data [58] from the RCTs has indicated a critical dose-response relationship between the magnitude of the convective volume and survival, with a goal of at least 23L per session. A Cochrane review suggests that there is no clear benefit of HDF over HD but this review combined outcomes of hemofiltration and HDF studies as convective therapies and did not assess outcomes based on convective volumes [71]. Improved cardiovascular outcomes in adults on HDF are, at least in part, explained by improved hemodynamic stability, leading to less intradialytic hypotension and faster recovery time after dialysis [56, 57, 69].

HDF Studies in Children

HDF is increasingly used in children in Europe but until recently there have been few data on outcomes. Fischbach and colleagues showed improved nutrition and growth [72], reduced inflammation [73], regression of left ventricular hypertrophy [73, 74], improved anemia control [73], and reduced postdialysis recovery time [72] in a small number of children undergoing daily HDF. HDF may have an anabolic effect associated with the greater removal of uremic toxins such as inflammatory cytokines and hormones that regulate appetite and growth, as well as superior clearance of accumulated endogenous somatomedin and gonadotropin inhibitors, improving target tissue sensitivity to growth hormone [75]. In a cross-over trial when HD patients were switched to HDF keeping all other dialysis related parameters constant, an improvement in inflammation, antioxidant capacity, and endothelial risk profile was achieved within 3 months [67].

A multicenter observational study tested the hypothesis that HDF improves the cardiovascular risk profile, growth and nutritional status, and health-related quality of life outcomes in children compared to conventional HD – the HDF, Hearts, and Height (3H) Study [70, 76]. Children receiving HDF had improved blood pressure and hemodynamic stability, reduced inflammatory markers, and lower β2-m compared to children on HD [70]. There was a very high prevalence of subclinical cardiovascular disease in children on dialysis, but an attenuated progression of carotid intima-media thickness increase was noted in children receiving HDF compared to those receiving conventional HD [70]. The annualized change in vascular measures correlated with improved BP control and clearances on HDF. Importantly, children treated with HDF reported a reduction in the frequency and/or severity of headaches, dizziness, and cramps on dialysis, as well as a reduction in the postdialysis recovery time, leading to an improvement in school attendance as well as physical activity [70]. Lower interdialytic weight gain on HDF, implying lower ultrafiltration rates per session and greater hemodynamic stability, was strongly associated with fewer symptoms. There was no reduction in serum albumin levels with HDF and no difference in the rate of change of residual renal function [70] in children on either dialysis modality, implying that HDF is a safe treatment.

Optimization of the Convective Volume

Every effort must be made to optimize the patient's convective volume to achieve the best outcomes from HDF. Convective volume may be improved by:

(i) ***Optimal vascular access.*** Both CVLs and AVFs can be used for HDF provided a good extracorporeal blood flow is achieved. The 3H study showed that it is possible to safely achieve high convection volumes (median 13.4 L/m^2) in children [76], which is comparable to the 23 L/1.73 m^2 session that proved beneficial in the adult studies [58], with no difference in the blood flow achieved through either type of access [70, 76]. With the exception of initial cannulation, one must use the largest AVF needle size suitable for the access type.

(ii) ***Optimal blood flow rates.*** The maximal blood flow rate that can be safely used will result in the optimal convective volume. The true blood flow rate may often be somewhat lower than the set value and the higher the blood pump speed, the wider the difference [77]. As blood flow rate increases, recirculation may occur, especially if there is inadequate arterial inflow or obstruction in the venous outflow; regular monitoring is required to avoid access recirculation.

(iii) ***Anticoagulation.*** Postdilution HDF may induce considerable hemoconcentration, so adequate anticoagulation is required. Unfractionated heparins have a molecular weight in the range of 2–20 kDa, so large convective volumes are likely to alter their pharmacokinetics [78], and higher doses than used with both low-flux and high-flux HD may be required [79, 80].

Home Hemodialysis

Home hemodialysis (HHD) was introduced in the early 1960s by groups in Boston, London UK, Seattle, and Hokkaidō, but was rapidly replaced in the 1970s and 1980s with the rise in hospital affiliated HD facilities. In the 1990s, as the demand for dialysis began to exceed the capacity within dialysis facilities, the interest in HHD returned. As HHD numbers started to increase, a new narrative began to unfold on the potential health and psychosocial benefits of HHD and practices started to expand worldwide.

HHD Definitions and Prescriptions

Home HD transfers dialysis treatments from hospital settings to patients' homes. One of the key characteristics of all HHD practices is augmented HD prescriptions, owing to a higher frequency of dialysis sessions per week or longer dialysis treatments. Typically, HHD regimens are subdivided according to their duration (treatment hours per session), frequency (alternate days or 4–7 times per week), and timing (daytime or overnight). One of the key drivers for families to transition to HHD is the flexibility to deliver the treatments around their own schedules.

HHD is prescribed as a weekly dose (standard Kt/V), individualized in partnership with the patient and their caregivers. Changes to the prescription are common and encouraged if an improvement in patient outcomes and/or quality of life is possible, balancing any decision against the potential burden of delivering care, as highlighted in Table 3.

Patient Selection Criteria

The referral for HHD is often initiated for medical, social, or educational reasons, or simply families exercising their right of choice. Failed home PD does not preclude the possibility of HHD. Criteria to consider include:

- A commitment to consistently delivering the dialysis schedule at home both by the patient and their caregivers.
- The patient weight cut-off criteria are determined by the choice of home dialysis system and equate to the combined volume of the extracorporeal circuit and dialyzer.
- Well-functioning vascular access.
- Absence of psychosocial concerns that cannot be adequately managed.
- Suitable accommodations that allow for the dialysis equipment and 2–4 weeks supply of dialysis consumables; adequate hygiene levels not to increase the patient's risk of infection.
- Ability to modify the water source if a reverse osmosis dialysis system is selected.
- The family does not live in an area with frequent and prolonged electricity supply disruptions or they have available an emergency source of power.
- Patient is medically stable even in the presence of possible multiorgan diseases.

Requirements for a HHD Program

Infrastructure Establishing a HHD program requires a central focus on safety. The service delivery model can vary considerably and is largely influenced by:

- Available resources and expertise
- Fully independent pediatric program or need to partner with a neighboring pediatric or adult program
- Dialysis regimens: Exclusively daytime schedules or include nocturnal treatments
- Dialysis system – consider dialysis consumables, deliveries, home modifications that may be required, service and maintenance for the dialysis machine and dialysis fluid system
- Training within the dialysis program or in a dedicated HHD training facility

Staffing and Remote Support It is crucial to recruit a multidisciplinary team to support HHD families, with a HD nurse, dialysis biomechanical engineer, nephrologist, dietitian, pharmacist, and social worker at minimum. It is essential to have a clear remote support structure for the family, so they understand how to access immediate help at any time. Patients should return to clinic regularly, every 4–8 weeks, and between clinics have regular contact with the HHD nurse specialist.

Training and Education Ideally it is advisable to train two people from the outset and involve patients to promote a degree of independence. All families should be offered repeated opportunities to enhance their knowledge and skills once home or following an adverse event or change in dialysis access such as switching from central line to AVF. Training can take place in the HD unit or within a separate training facility.

Table 3 Essential considerations when selecting the most appropriate pediatric HHD prescription

Prescription	Session duration [h]	Sessions per week	Patient or family considerations	Prescription considerations
Short daily	2–3	6–7	Helpful for working/busy parents with a limited window for dialyzing Higher frequency best for children with high UF requirements such as enterally fed infants or children poorly adherent to fluid restrictions.	Most expensive option due to higher dialysis consumables cost Dialysis and blood flow rates are typically unchanged from in-center prescriptions 2 h prescriptions not suitable for infants as the extracorporeal circuit volume becomes a significant additional UF burden on top of the interdialytic weight gain Dietary and fluid restrictions often unchanged
Extended	4.5–6	Alternate days at minimum to 7 days per week maximum	Alternate-day therapy offers greatest respite time for the caregiver Teenagers become increasingly frustrated sacrificing their evenings to HD Longer duration best for children with intradialytic symptoms and cardiac instability	Dialysate and blood flow rates typically 15%–30% lower than in-center prescription May require higher dialysate calcium requirement, especially infants and teenagers with higher growth rates Often allows liberalization of dietary and fluid restrictions Improved BP control, lower antihypertensive requirement
Nocturnal	7–12	Alternate days at minimum to 7 days per week maximum	Dialyzing overnight can induce anxiety in caregivers and children from fear of disconnection or unattended machine alarms Virtually eliminates adverse intradialytic symptoms Greatest chance of achieving complete freedom from dietary and fluid restrictions possible Best for children with cardiac disease	Requires additional safety considerations Dialyze against 1.75 mmol/L dialysate calcium to protect against a negative calcium balance Higher frequency nocturnal HD may cause hypophosphatemia. Treat with oral supplements and/or add phosphate to the dialysate concentrate as a sodium phosphate enema Theoretical risk of “dialysis deficiency syndrome” (i.e., nonselective purification of essential plasma components). Consider surveillance and replacement of vitamins and minerals

These considerations are based on personal opinion
BP blood pressure, *HD* hemodialysis, *UF* ultrafiltration

Regardless, prior to going home, families should step down to an environment separate from the dialysis unit, in order to simulate the home environment as much as possible. Companies supplying the dialysis equipment will often provide considerable teaching materials and expertise, but invariably pediatric adaptation is necessary. Prior to discharge, an adherence contract signed by the child and their caregivers can be extremely helpful in formalizing the expectations and for reinforcing that any deviation will be taken very seriously.

Dialysis Equipment Most HD machines that are suitable for HHD require home water conversions to produce the large volumes of high-quality dialysate necessary for the dialysis treatment. Water conversion requires the installation of a cold-water outlet and a drain to allow the carbon filter, reverse osmosis unit, and dialysis machine to be fitted. A system for testing the water quality needs to be put in place. Typically, families test their water for chlorine and chloramine every session and then bring a sample for advanced testing to their monthly hospital clinic visits. The dialysis technician will test for chemicals, endotoxins, and microbiology.

The NxStage System One™ (NxStage Medical Inc., Lawrence, MA, USA) is a portable home dialysis machine that functions without home water modifications. Dialysis fluid is prepared in batches up to 60 l using the NxStage PureFlow™ SL integrated water purification and dialysis fluid production system, which uses ordinary tap water mixed with sterile-filtered concentrate to produce lactate buffered dialysis fluid. Premixed dialysis fluid is also available for home patients in sterile, 5L bags, with a variety of fluid compositions.

Potential Advantages of HHD over Conventional HD

HHD prescriptions have the potential to deliver a higher dialysis dose and allow for lower UF rates. The dialysis dose is important in lower body mass index (BMI) patients such as children. The HEMO study demonstrated a higher mortality risk in patients with the lowest BMI [81]. Increasing the dialysis dose as reflected by a higher urea reduction provided a greater survival advantage in the medium and small BMI groups [82]. The DOPPS review of 22,000 HD patients found that a higher dialysis dose, indicated by a higher Kt/V, was an independent predictor of lower mortality with a synergistic survival advantage with treatment time. Therefore, survival was most pronounced by combining a higher Kt/V with longer treatment time, such that for every 30 min longer on HD the relative risk of mortality was reduced by 7% [83]. An ANZDATA analysis found that the optimal dialysis dose for survival was a Kt/V greater or equal to 1.3 and a dialysis session greater or equal to 4.5 h. A single dialysis session prescribed for less than 3.5 h was associated with a higher mortality risk [84]; also see ▶ Chap. 78, “Adequacy of Dialysis.”

The UF rate during dialysis is also an important and significant factor in mortality. Movilli and colleagues demonstrated a critical safe point, with better survival with UF rates less than 12.37 mls/kg/h [85]. Patients who frequently exhibit intradialytic hypotension are at a higher risk of mortality compared to those who experience no hypotension [86]. Longer or more frequent dialysis sessions can prevent the deleterious consequences of excessive UFR or chronic hypervolemia.

Studies in Adults The majority of the literature on HHD is from observational or longitudinal studies, with only two randomized controlled studies to date [87, 88]. Reported improvements include reduced antihypertensive medication burden and lower incidence of left ventricular hypertrophy; improved anemia control and a lower average erythropoietin dose; a reduction in the use of phosphate binders and improved nutrition; and improved quality of life. During a matched cohort study comparing survival between nocturnal HD and deceased and living donor kidney transplantation, the proportion of deaths among the three was 14.7% for nocturnal HD, 14.3% for deceased donor transplantation, and 8.5% for living donor transplantation [89]. In a comparison with peritoneal dialysis, patients reported a similar perception of control over their kidney disease and did not consider HHD as a more intrusive treatment [90]. A meta-analysis of 681 pregnancies showed an inverse relationship between hours of dialysis and the risk of a preterm delivery or a baby born small for gestational age [91].

Studies in Children Pediatric data are scarce with mostly single-center reports; findings are largely consistent with the adult experiences. Short, 6 times per week HHD sessions resulted in reductions in BP and improved serum phosphate levels without changes to phosphate binder doses [2]. Dialyzing 5 h, 3 times per week, with a caloric intake of 150% of the recommended daily allowance for age led to improved growth [92]. Nocturnal HD, 6 nights per week, in four children age 10–19 years, achieved a weekly Kt/V of 7.2–13.6, fluid and dietary restrictions were completely liberalized, phosphate supplements were required to avoid hypophosphatemia, catch-up growth was achieved, and quality of life improved markedly [93]. Similar results were reported on six children age 11–17 years on nocturnal HD. BP control was variable, with two patients remaining on antihypertensives, and three patients requiring prophylactic midodrine to support their BP during UF [94]. All were dialyzed against 1.75 mmol/l calcium to prevent a net negative calcium balance [95]. Appetite, well-being, school attendance, physical and psychological health-related quality of life scores improved in all patients. However, caregivers' feedback highlighted the perceived burden and anxiety of dialyzing at home. The mother of one patient was psychologically and emotionally worn out after 1 year, necessitating respite arrangements of once weekly in-center treatments [94]. Finally a case of a 10 year old with presumed severe, irreversible cardiomyopathy treated with extended and then nocturnal HHD, reported completely normal cardiac function after 6 months of HHD [96]. It is postulated that the superior purification with minimal cardiovascular instability during UF was conducive to stopping and even reversing dialysis associated cardiac injury that we have come to accept from conventional HD prescriptions.

Risks and Complications Related to the Hemodialysis Procedure

While the approach to HD is well established and HD technology continues to advance, the HD procedure itself carries risk. Careful monitoring with safety devices and observation combine to assure a safe HD session. Despite safety as a top priority in all dialysis programs, patients may experience complications from the HD procedure.

Intradialytic Hypotension

Intradialytic hypotension is common, driven by complex pathophysiology including impaired hemodynamic compensatory responses with ineffective venoconstriction in the uremic milieu, reduced plasma refilling, a suboptimal metabolic stress response, inadequate ventricular refilling, lower myocardial contractile reserve, and impaired resting baroreflex sensitivity. In children the volume of blood within the extracorporeal circuit can be a significant proportion of the circulating volume. Tachycardia for any reason during the HD treatment will reduce diastolic ventricular filling and reduce myocardial perfusion. Blood and dialyzer membrane inflammatory reactions can result in early decompensation.

Noninvasive blood volume monitors provide a measure of the changes in relative blood volume (RBV), real-time during dialysis treatments. Several studies in adults have failed to demonstrate a critical threshold that can predict adverse events but found that irregularity of the RBV course, gradient of the RBV curve, and switching from an exponential to a linear RBV reduction were the most powerful predictors of intradialytic hypotension [97–99]. Likewise in children, the point of decompensation showed large intrapatient and interpatient variability; changes in heart rate and a steeper gradient of the RBV curve in the first hour were predictive of hemodynamic decompensation [50].

Consequences of Intradialytic Hypotension Intradialytic hypotension can cause symptoms that may become intolerable or severe such as cramps, headaches, and loss of consciousness. In adult dialysis patients, a "J" shaped curve exists between BP and mortality [100], with a fourfold increase in the relative risk of cardiac-related death in patients with predialysis systolic BP less than 110 mmHg compared with a systolic BP between 140 and 149 mmHg, and a 2.8-fold increase in relative risk for a cardiac-related death with post dialysis systolic BP less than 110 mmHg compared with systolic BP 140–149 mmHg [101]. Repeated intradialytic hypotension can result in recurring episodes of ischemia, accelerating the decline in residual renal function and multiorgan ischemic injury. In the heart, myocardial ischemia causes reversible, regional, left ventricular dysfunction, a phenomenon called myocardial stunning, with further intradialytic cardiovascular instability. Myocardial stunning has been demonstrated in children during conventional HD and the degree of myocardial dysfunction correlated with the UF rate and intradialytic blood pressure change [102].

Management of Intradialytic Hypotension Acutely, intradialytic hypotension requires immediate stepwise action dependent on the severity. This may include temporary suspension of the UF, providing a 5 ml/kg isotonic fluid bolus and early discontinuation of the dialysis treatment. Hemodynamic stability during dialysis can be improved by withholding antihypertensive medications on dialysis days, avoiding food during dialysis, and cooling the dialysate temperature to at least 0.5 °C below the patient's temperature prior to starting dialysis. UF profiles (decreasing step, decreasing linear, and alternating high/low UF rate) have been shown to be no better than a constant UF rate in preventing intradialytic symptoms or hypotensive episodes in children [103]. Manipulating the dialysis fluid composition can also improve hemodynamic stability:

- Switching to bicarbonate buffers compared with acetate buffers.
- Monitoring the plasma calcium levels and adjusting the dialysis fluid calcium concentration to avoid intradialytic hypocalcemia.
- Adjusting or profiling the dialysis fluid potassium concentration to avoid large potassium gradient changes during dialysis.
- Sodium profiling, utilizing a sodium concentration that falls in a step, linear, or exponential fashion. The higher dialysis fluid sodium counterbalances the rapid decline in plasma osmolarity due to clearance of urea and other small molecular weight solutes thus promoting plasma refilling. The low dialysis fluid sodium at the end minimizes the adverse effects of sodium loading.

Managing intradialytic hypotension will often require a combination of these interventions as opposed to trialing them in succession. In resistant cases, midodrine is a specific α-1 adrenergic agonist that mediates both arterial and venous constriction; it can prevent venous pooling while supporting central BP. Alternatively switching to HDF or augmented dialysis prescriptions may be required.

Dialysis Disequilibrium Syndrome

The disequilibrium syndrome is characterized by neurological changes, seizures, and possible risk for mortality in association with HD [104]. The reverse osmolar hypothesis suggests that disequilibrium occurs in the setting of rapid clearance of small molecules when the starting blood level of urea is very high. Rapid removal of urea from the blood causes a transient osmolar gradient for urea across the blood-brain barrier, drawing water into brain cells and leading to acute cerebral edema [105]. Since the disequilibrium syndrome appears associated with higher rates of dialytic clearance, the likelihood of this complication diminishes with lower rates of clearance, particularly for the first few HD sessions. Other maneuvers include giving infusions of mannitol during the HD session to compensate for the removal of other osmotically active particles (urea).

Fever and Infection

Fever in a HD patient raises concern for blood stream infection. This is more likely if the patient has an indwelling CVL. Not all patients with HD-associated infection will have fever and not all febrile HD patients will have a serious infection. The dialysis team should maintain a high index of suspicion and a low threshold to obtain cultures and provide antibiotics, at least until blood cultures results are obtained.

Vascular Access Related Infection

The presence of the foreign body that is the CVL, with passage through the skin, increases risk for infection. There are several potential infectious complications related to HD catheter use, of varying severity. Minor infections without systemic findings may be treated successfully with brief courses of antibiotics. More serious or recalcitrant infections will obligate the use of parenteral antibiotics for longer courses and, if the patient is at increased risk or the clinical status is not improving or is worsening, may necessitate removal of the HD catheter. Infection associated with AVF or AVG is much less common.

Catheter Exit Site Infection Despite appropriate local care at the exit site, the CVL predisposes to infection. Exit site infection commonly presents with pain, redness, or swelling at the exit site and drainage, possibly purulent. Exit site infection is less likely to present with concomitant fever; if the patient is febrile, one must have a higher index of suspicion for systemic rather than localized infection. The most common infectious agents causing exit site infection are skin flora, most often gram-positive bacteria such as *Staphylococcus* species. Swab sampling of any purulent drainage, sent for culture and gram stain, will help to clarify the specific bacterium and may guide therapy. Presumptive antibiotics would cover for the likely organisms, with choices adjusted depending on common local epidemiology and patient-specific history. In the absence of systemic findings and a judgment that the infection is superficial and localized to the exit site, oral antibiotics are a reasonable choice. Common options may include first-generation cephalosporin or clindamycin. Oral therapy can be adjusted based on culture results.

Catheter Tunnel Infection With a presentation similar to exit-site infection, HD catheter tunnel infection usually also demonstrates pain/tenderness along the track of the catheter itself, often with erythema and swelling. The patient may report warmth of the skin around the catheter and expanding area of redness. Fever is more common with tunnel infection than with infection localized to the exit site. Tunnel infection puts the patient at increased risk for bacteremia; parenteral antibiotics, providing coverage for gram-positive bacteria, should be employed and given the challenges of successfully clearing an infection around the foreign body, HD catheter removal may be required.

Catheter-Associated Blood Stream Infection CVLs are associated with the majority of bloodstream infections in HD patients [106]; bacteremia is roughly ten times more common in patients undergoing HD with a CVL compared to those with AVF or AVG. This is associated with greatly increased risk of mortality for those HD patients who develop sepsis, reported at 100 to 300-fold higher than for those in the general population [107]. Catheter-associated infection is more commonly seen with temporary HD catheters than with tunneled catheters; [108] the external cuff of the tunneled catheter serves as a barrier to penetrating infection and the length of the catheter tunnel also limits bacterial entry.

The majority of blood stream infections in HD patients, possibly up to 80%, result from gram-positive cocci such as coagulase-negative staphylococci or *S. aureus*, but gram-negative and polymicrobial infections are also seen [106]. Patients may present with fever, chills, general malaise or may have unexpected blood pressure instability during the HD session. There should be low threshold to obtain blood cultures and promptly begin parenteral antibiotic treatment. After obtaining blood cultures, most HD programs will empirically treat with broad-spectrum antibiotics to cover for both gram-positive and gram-negative bacteria; options include vancomycin and gentamicin or ceftazidime. Care is required when choosing antibiotics given the potentially negative impact of nephrotoxic medications on residual renal function, balanced with the necessity of treating a patient at high risk for complications. Once the infectious agent is identified and antimicrobial sensitivity pattern is available, one may narrow antibiotic therapy to a more specific option.

Many HD programs will add antibiotic locking solution to the regimen; antibiotic lock of the HD catheter will improve killing of bacteria inhabiting the biofilm within the HD catheter lumen. Antibiotic lock solutions blend heparin with an antibiotic such as vancomycin, ceftazidime, or cefazolin. The solution is instilled to the catheter lumen at the end of each HD session and can continue until the course of systemic antibiotic therapy ends or the HD catheter is removed.

Ideally, any infected venous catheter would be removed to permit clearance of the infection. In HD patients, removal and replacement of the vascular access is complicated by limited sites for replacement and the ongoing requirement for access to perform the HD sessions. Many infections can be cleared successfully with antibiotics, but in some cases the HD catheter must be removed. If the patient has evidence for sepsis, source control of infection by urgent catheter removal may be necessary. Patients with concomitant exit site and/or tunnel infection usually require catheter removal to fully clear the infection, even with less severe clinical signs and symptoms. Persistent fever/symptoms, or infection with agents that are known to be challenging to clear (e.g., *S. aureus*, *Candida* species, polymicrobial infections, resistant organisms), likely also will require catheter removal. After catheter removal, one must consider options for replacement to permit ongoing HD; this requires review of blood culture clearance, consideration of length of antibiotic treatment, clinical status of the patient, residual renal function, and alternative access sites for catheter re-insertion. For patients with persistently positive blood cultures, one may have to consider placement of a temporary catheter for HD; such a catheter could be removed more easily should it become re-infected prior to clearance of the bacteremia. Some patients may require catheter removal but are stable clinically and have evidence of having cleared their infection based on negative cultures. For such patients, one may consider catheter exchange over a guide wire. This approach preserves overall sites for vascular access by re-using the current access location and maintains continuity of access to avoid skipped HD session. Many adult HD programs have adopted catheter exchange over wire protocols for all stable patients with blood stream infection, noting increased rates of cure [109]. This approach may be more challenging in children for whom vascular access options are limited.

Infection Associated with AVF or AVG Compared to HD catheters, infections in patients who dialyze with AVF or AVG are infrequent [110]. While the rate is much lower, the infectious agents are similar. Risk increases in those patients with pseudoaneurysm, injury, or inappropriate puncture (e.g., injection of drugs of abuse). The button-hole technique for access puncture has also been associated with a higher rate of infection but not in all populations [40]. Given the lower likelihood of infection related to the access itself, as compared to those with HD catheter, a patient with evidence for bacteremia who dialyzes with AVF or AVG may be more likely to have endocarditis; consequently, extended course of antibiotics is recommended [111].

Blood Borne Virus Infections HD patients are at increased risk for infectious hepatitis. Of greatest concern is infection with hepatitis B or C; HD patients suffer increased morbidity and mortality with these infections related to the underlying immunosuppression of ESKD. Hepatitis is a significant infection control risk; HD units must engage in screening and appropriate isolation while also maintaining universal precautions to prevent transmission from undiagnosed patients.

Elevated Blood Pressure

High blood pressure is common in the HD unit [112]. Overwhelmingly, high blood pressure in a patient on maintenance HD stems from volume excess and improves with salt and fluid removal. Assessment of the volume status in a child on dialysis is described in detail in ▶ Chap. 74, "Assessment of Volume Status in Chronic Kidney Disease." Chronic hypertension requires a controlled and gradual lowering of the target weight and dietary sodium restriction. Persistent high blood pressure, after convincing efforts to reduce total body water and sodium excess, especially in those dialysis patients who retain their kidneys, may suggest renin-mediated hypertension. Such patients may benefit from renin-angiotensin-aldosterone system blockade, using angiotensin converting enzyme inhibitors, along with ongoing sodium/volume control.

Hemorrhage

Bleeding associated with HD relates to extracorporeal perfusion and the presence of a vascular access. Disconnection of the blood circuit tubing set from the vascular access puts the patient at risk for hemorrhage during the dialysis procedure. Monitors on the dialysis machine note the sudden change in pressure within the blood circuit at the time of disconnection and stop the blood pump while sounding an alarm; this greatly reduces the chances of rapid exsanguination which could occur with a venous limb disconnection and continued blood pump rotation. A more common scenario for blood loss in the dialysis unit occurs with clotting of the blood circuit during the session or mechanical failure of the hemodialysis device, after which bedside staff cannot return the volume of blood in the extracorporeal circuit to the patient. Careful assessment of the patient may show symptoms related to volume loss requiring volume expansion with crystalloid or, in severe cases, blood transfusion; if the patient is asymptomatic, treatment is expectant.

Air Embolism

Air embolism is a potentially serious complication in which a breach of the blood circuit, most commonly at a connection site, entrains air into the blood flow system that then enters the patient [113]. This can lead to acute respiratory compromise similar to a thrombotic embolism. Acute management includes immediate cessation of the dialysis procedure, 100% oxygen to the patient, and critical care support if indicated. The air detection system on modern dialysis equipment makes air embolism very rare, but its seriousness requires all dialysis providers to have a high index of suspicion.

Water-Related Complications

Contamination of a dialysis unit water system, with either microbes or biochemical contaminants, can have devastating effects on multiple patients simultaneously. For example, chloramine, a by-product of chlorine, when not appropriately removed from dialysis water, can cause severe hemolytic reactions and death [114]. Given this risk, dialysis staff must check for the presence of chloramine, which the water treatment system normally removes quite effectively, multiple times a day. The entire dialysis water system must undergo frequent monitoring and ongoing preventive maintenance to assure its integrity and the safety of the patients.

Intradialytic Cramping

Cramping during the HD session is a common patient complaint. It appears to be associated with higher rates of UF and may therefore be related to reduced muscle perfusion. In addition, intradialytic electrolyte imbalances and changes may also be a cause. Some report that abdominal cramping is more common when patients eat or drink during dialysis sessions. Preventive measures, such as working with patients to limit interdialytic weight gain leading to the need for high rates of UF, may be the best approach. Medications such as quinine, used more commonly for adults, have fallen out of favor due to side effects. Sodium modeling may limit muscle cramping on dialysis [115] but may also limit negative sodium balance during the session, putting the patient at risk for volume overload and high blood pressure [116, 117].

Long-Term Outcomes of Chronic Hemodialysis in Children

While advances in dialysis technology have improved outcomes even for the youngest children, mortality remains high and the morbidity and burden of care cannot be underestimated. Optimal management of children on dialysis requires a multidisciplinary team and consideration of the child and family's expectations. Most importantly, minimizing the time on dialysis by early or even preemptive renal transplantation is currently the best option for ESKD patients.

With few high-quality randomized trials, clinicians are reliant on prospective cohort studies and international registries to inform and improve management strategies.

Cardiovascular Disease and Survival

Children on HD have a mortality rate that is 30-fold higher than their age-related healthy peers [118], and cardiovascular disease accounts for over 20% of deaths. Cardiovascular events occur in approximately 25–35% of children on chronic HD and include arrhythmias, valvular heart disease, cardiomyopathy, and sudden death [119]. Left ventricular hypertrophy (LVH) has been reported in 40–75% of children on maintenance dialysis [120]. Risk factors include chronic hypervolemia, a hyperdynamic circulation secondary to AVFs or anemia, and vascular calcification leading to increased arterial stiffness [121]. Cardiac hypertrophy and continued mechanical stress can trigger myocardial remodeling and reduce coronary flow reserve predisposing the heart and other organs to ischemic injury.

Vascular and valvular calcification can begin in CKD but is more prevalent in dialysis patients, with an accelerated progression of calcification that increases with time on dialysis [119]. Coronary calcification has been reported in 20–30% of children on dialysis. While traditional cardiovascular risk factors such as dyslipidemia and inflammation are associated with intimal disease or atherosclerosis [122], the uremia-related risks, in particular dysregulated mineral homeostasis including high calcium, phosphate, PTH, and vitamin D as well medications used to treat MBD including calcium-based phosphate binders and vitamin D, are associated with medial calcification or atherosclerosis [123–125]. The etiology and consequences of vascular calcification are discussed in detail in ▶ Chap. 72, "Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD)." Atherosclerosis can lead to increased vessel stiffness, high systolic blood pressure, left ventricular hypertrophy, and reduced coronary blood flow. Improving phosphate clearance by using intensified dialysis regimens is shown to reduce the prevalence of calcification and LVH [73, 126].

Nutrition, Growth, and Development

Malnutrition and poor growth are seen in children on HD, particularly if they have been underdialyzed, and have been associated with mortality. There is a 30–50% risk of short stature in children on chronic dialysis. [127, 128] Mechanisms of poor growth include reduced appetite due to dysregulation of the hormones regulating appetite and satiety and resistance to growth hormone. Nutrition in CKD and dialysis is discussed in detail in ▶ Chap. 71, "Nutrition, Growth, and Development." The normalized protein catabolic rate (nPCR), an indirect measure of daily protein intake in stable dialysis patients, and derived from formal urea kinetic modeling or calculated from blood urea nitrogen (BUN) measurements [127], can be used to guide protein intake; details discussed in ▶ Chap. 71, "Nutrition, Growth, and Development." However, it may be influenced by factors other than nutrient intake [129] and should be interpreted in combination with a review of weight gain and the dietary history [130].

Children with CKD may have low-average neurocognitive and academic outcomes, and cognition appears to worsen with advancing CKD, with children on dialysis having the poorest performance [131]. Neurocognitive disorders in CKD are discussed in detail in ▶ Chap. 71, "Nutrition, Growth, and Development."

Impaired Health-Related Quality of Life (QoL)

Children on dialysis are exposed to treatment regimens that are long-term, complex, and highly intrusive. They may suffer from fatigue, sleep disturbances and pruritus, may require multiple hospital admissions, and have long-term dietary and fluid restrictions, all of which can disrupt home and school life, reduce the child's social contact with their peers and families, and contribute to a poor QoL [132–134]. Children with CKD are at risk of cognitive impairment and poorer educational, vocational, and psychosocial outcomes compared with their well peers, which have consequences through to adulthood [135]. In the longer term, young adults who had received dialysis during their childhood were more likely to be unemployed, and less likely to be in a long-term relationship [136]. The Standardized Outcomes in Nephrology (SONG) initiative recognized that survival and "life participation" were important treatment goals for children [137], stressing the importance of working in partnership with the child and family to help them achieve an optimal QoL.

Other Long-Term Complications

Dialysis Related Amyloidosis Amyloid accumulation is a disabling, progressive condition caused by amyloid accumulation predominantly in the osteoarticular systems that can lead to carpal tunnel syndrome and spondyloarthropathy and accumulation in the vessel walls and heart contributing to uremic cardiovascular disease. It is primarily related to the accumulation of β2-microgolbulin, a middle molecular weight (11.6 kDa) protein that is primarily cleared by convection. Hence, it tends to accumulate in patients on conventional HD but is effectively removed by HDF, providing around 70% better removal than conventional HD [70].

Dialysis-Related Carnitine Disorder Levocarnitine (L-carnitine), a critical co-factor for normal energy production in cardiac and skeletal muscle, is rapidly removed by HD [138] resulting in an abnormal acylcarnitine: free carnitine ratio. Low carnitine levels may be associated with intradialytic hypotension, cardiac dysfunction, fatigue, muscle cramping, reduced exercise tolerance, and anemia [139]. L-carnitine supplementation is recommended for patients with clinical symptoms even in the absence of low plasma carnitine levels [140].

Hyperhomocysteinemia Homocysteine, a non-protein forming amino acid that results from methionine metabolism, accumulates from early stages of CKD. A meta-analysis reported a positive association between hyperhomocysteinemia and atherosclerosis, ischemic heart disease, stroke, and thrombosis [141], but others have found no significant or even an inverse association between plasma homocysteine levels, cardiovascular events, and mortality in ESKD patients [142]. Treatment options include folate and vitamin B12 supplementation to achieve supranormal plasma levels, or intravenous N-acetylcysteine, [143] or improved clearance with high-flux dialyzers [144].

Conclusion

Technical advances have facilitated chronic HD treatment in even the youngest children, and improvements in medical and nursing skills have made HD safer and more effective than ever before. Intensified HD with extended hours, home HD as well as in-center HDF have improved outcomes compared to conventional HD regimens. Nevertheless, children on HD have a high morbidity and mortality, accruing a significant burden of cardiovascular risk factors. Thus, even as efforts continue to improve HD technology, nephrologists must aim for preemptive kidney transplants where possible, using dialysis only as a bridge to transplantation.

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Infant Dialysis

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Abstract

Infant dialysis remains one of the greatest challenges in pediatric nephrology due to the difficulties encountered during the management of patients of small size, and the need for complex and costly resources in order to provide good medical care. There are number of factors which make infant dialysis special. Of importance is that kidney failure in the first months of life is in most cases associated with a variety of other anomalies or comorbid conditions. The severity of the accompanying conditions impacts the likelihood of survival and influences the decision whether to initiate dialysis. Once the decision has been made to start dialysis, issues of growth and development, infectious and noninfectious dialysis complications, quality of life, and preparation for transplantation need to be addressed. One of the key treatment challenges is providing optimal nutrition as it is the predominant factor affecting growth, psychomotor, and cognitive development in infancy. Peritoneal dialysis (PD) remains the modality of choice prescribed to infants worldwide, due to its simplicity, avoidance of vascular access, partial independency of hospital setting, better preservation of residual kidney function, and fewer dietary and fluid restrictions. If PD is contraindicated or not feasible, hemodialysis is also a successful option.

Keywords

Infant dialysis · Epidemiology · Ethics · Nutrition · Growth · CKD-MBD · Long-term outcome

Introduction

Advances in peritoneal dialysis (PD), hemodialysis (HD), and kidney transplantation over the decades mean that it is possible to treat even infants with end-stage kidney disease (ESKD) [1]. However, this comes at a cost because infants demonstrate higher rates of hospitalization, morbidity, and mortality when compared to older children on dialysis [2]. Kidney transplantation is the preferred kidney replacement therapy (KRT) for all children with ESKD; however, since kidney transplantation in infants is technically limited by patient and vascular size, all infants with ESKD need chronic dialysis until they reach approximately 10 kg in body weight [3]. Impaired growth due to inadequate nutrition, poorly controlled CKD-mineral bone disorder (CKD-MBD), and other ESKD-associated conditions remain major clinical issues in infants on dialysis [4, 5, 6]. Furthermore, there are ethical debates regarding the initiation and maintenance of chronic dialysis in ESKD infants with complex comorbidities [7]. Thus, infant dialysis presents one of the greatest challenges in the field of pediatric nephrology (Table 1).

This chapter will address the current knowledge on the epidemiology, ethics, when to start and stop dialysis, approaches to PD and HD, growth and nutrition, management of CKD-MBD, and long-term outcomes of infants requiring chronic dialysis therapy.

Table 1 Challenges in infant dialysis

More than 50% are diagnosed antenatally
Prematurity or low birth weight common
Decisions may be needed regarding commencement or withholding of dialysis
Pre-emptive transplantation is not possible
Preservation of access sites is a particular problem
Feeding and growth:
Need to support a high nutritional requirements and growth rate
Liquid diet affects dialysis program
Vomiting common
Enteral feeding frequently required
Calcium requirements high
Salt depletion may be an issue
Burden of care
Limited clinical experience in most centers
No evidence-based studies

Epidemiology

Number of Infants Initiating Chronic Dialysis

Before the early-1980s long-term outpatient dialysis could not be undertaken in infants with ESKD [8]. However, improvements in dialysis technology and clinical expertise mean that this is now a practical option for most infants. Based on registry reports from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) and the European Society of Pediatric Nephrology and European Renal Association-European Dialysis and Transplant Association (ESPN/ERA-EDTA), the numbers of infants initiating chronic dialysis were 901 from 1992 to 2012 and 1063 from 1991 to 2013, respectively [9, 10]. Furthermore, a report from the NAPRTCS registry indicated that the proportion of infants initiating chronic dialysis increased from 13.4% in the period of 1992–2001 to 14.9% in the period of 2002–2011 [9]. A similar trend was also observed in data from the United States Renal Data System (USRDS) registry. The numbers of infants initiating chronic PD were 543 from 1990 to 1999 and 1180 from 2000 to 2014, respectively [11]. Thus, the number of infants initiating chronic dialysis has recently increased in high-income countries.

However, health disparities in access to KRT among children with ESKD still exist between children of low- and lower-middle-income countries as compared to higher-income ones [12]. Since infants with ESKD are more medically complex and need more resources, the number of infants initiating chronic dialysis is largely influenced by economics: the International Pediatric Peritoneal Dialysis Network (IPPN) reported that the age distribution of the children undergoing chronic dialysis markedly varied with economic wealth (expressed as per-capita gross national income), with almost 1 of 5 patients in the high-income countries, but only 1 in 50 in the low-income counties, being younger than 12 months of age (Table 2) [13]. Analyses of European middle-income and high-income countries showed that there were differences between countries in their ability to accept and treat infant ESKD patients, which were largely explained by differences in public health expenditure [14]. Thus, the number of infant initiating chronic dialysis is strongly affected by a country's gross national income and public health expenditure.

Primary Diagnoses and Comorbidities

Based on registry data from NAPRTCS, ESPN/ERA-EDTA, and USRDS, the most common primary diagnosis in infants requiring chronic dialysis therapy is CAKUT (including kidney hypoplasia/dysplasia and obstructive uropathy), comprising around half the cohort [9, 10, 11]. Other causes are cystic kidney disease (mainly, autosomal recessive polycystic kidney disease), neonatal cortical necrosis, congenital nephrotic syndrome, kidney venous thrombosis, hemolytic uremic syndrome, primary hyperoxaluria type 1, and angiotensin-receptor blockade fetopathy [11, 15]. Thus, causes of ESKD in infants differ in comparison to older children with ESKD [16].

Comorbidity is particularly common in this age group, with a reported incidence of up to 75%

Table 2 Gross national income and age at start of dialysis in chronic peritoneal dialysis patients who were enrolled in the IPPN registry between April 2007 and March 2012

Age at start of dialysis (%)	Gross national income in $000s per capita			
	>28	18-28	12-18	<12
<1 years	19	18	16	2
1-3 years	11	15	11	6
3-6 years	8	14	10	16
6-12 years	21	28	35	38
12-18 years	37	23	28	37

Adapted from Ref. [13] with modifications

depending on the population studied and how comorbidity is defined. Syndromes, chromosomal abnormalities, developmental delay, cardiovascular disorders, lung abnormalities including pulmonary hypoplasia, neurological disorders, and liver disease are the commonest [14, 15, 17]. Thus, many infants requiring chronic dialysis therapy also require care from many medical teams because of their complex conditions.

The Ethics of Infant Dialysis

Advances in resources to diagnose abnormalities in utero have outstripped our ability to use the information to predict fetal and neonatal outcomes. Ultrasound (US) can detect the majority of cases of congenital abnormalities of the kidneys and urinary tract (CAKUT), which occur in 1–4 per 1000 pregnancies. Most of these are benign. US can also detect cystic kidney diseases. Genetic studies may be helpful for diagnosis, although rarely so for CAKUT unless there are associated comorbidities. Although kidney sizes and appearances, bladder pathology and amniotic fluid volume may be used to counsel families as to the potential that their child will need KRT in the future, there is no infallible method that enables clinicians to be certain whether this is likely to be an immediate requirement or one that is many years away [18].

For these reasons, the perinatal period is a time that is fraught with anxieties and difficult decisions for the family. Miscarriage may occur with severe kidney abnormalities but the incidence of this is unknown. Antenatal surgery has been proposed but its success is variable and unproven [19] Termination may be discussed in those pregnancies that are thought by the managing team to be at very high risk. The family, in these circumstances, will have to live through months of uncertainties, and will have expectations for their infant that may or may not be realistic [20].

As well as parental preconceptions (which may be correct or incorrect) about the future for their infant and management after birth, there are factors that may bias the attitudes of the medical team as well, and lead to ethical issues. For the infant needing KRT, a decision may need to be made about its appropriateness. Many infants have associated comorbidities, and these, together with uncertainty for individual outcome, carer burden and resources, can contribute to difficult decision making.

The European Paediatric Dialysis Working Group (EPDWG) addressed these issues and suggest in their guidelines that comorbidities and predicted future quality of life for the infant and their family; availability of resources, both medical and those of the family support structure; and prognosis and possibility for future transplantation should be taken into consideration when deciding whether to go forward with dialysis. The parents should be actively involved in the decision-making process [21].

Countries have produced guidelines to help the multidisciplinary team in determining the management of severely ill children, and these apply equally to all causes of illness. The overriding principle is that the current and future treatments must be “in the child’s best interests,” i.e., that benefits outweigh burdens; any potential harm is minimized; and the child is treated fairly and justly. Future quality of life, which has been defined as survival without life support, life outside of hospital, ability to develop relationships, and ability to gain pleasure from life is deemed more important than chances of survival. There is no right or wrong answer to these dilemmas, and the multidisciplinary team must work with the family to obtain a satisfactory outcome [7].

When to Start Dialysis

The majority of infants with CKD are affected by CAKUT. As abnormal tubular function predominates over glomerular dysfunction, most infants are salt and water losers and continue to pass urine, even in large quantities, in the face of very low Glomerular Filtration Rates (GFRs). There are some infants who do not pass urine, such as those with renal agenesis or renal tubular dysgenesis, when dialysis is clearly needed to prolong life. However, where there is adequate urine, good dietary input and medical therapy can enable the infant to thrive, with good metabolic control for extended periods [22]. However, given the rarity of such severely affected infants, many teams will not have the experience or resources, particularly pediatric kidney dieticians, to manage these infants conservatively.

There are factors that help in prediction of the potential for kidney recovery. Many infants with CAKUT are premature or of low birth weight, with the potential for damage to the ongoing development of the glomeruli as a result [23]. There may be further insult to the kidney from obstruction or UTI; or from respiratory compromise due to lung hypoplasia. On the other hand, as blood flow to the kidney improves and kidney maturation continues, this may be overridden by a steady improvement in kidney function.

The improvement in GFR that is seen in the first few weeks of life may be enough to keep the infant from dialysis until they have grown sufficiently to postpone the technical difficulties associated with small size. This is substantiated by European data that shows that of 238 neonates commencing dialysis in the first month of life, 19 (8% of all neonates) were able to stop it. When a nadir in creatinine is reached, a decision can be made about the need for dialysis to preserve metabolic control, fluid balance, and growth [24].

A key management point is to ensure adequate salt and water administration. Dehydration and poor kidney perfusion is a frequent accompaniment of CAKUT, and the temptation to restrict both salt and water must be avoided, even with very low GFRs in these infants [25].

Choice of PD/HD

Although kidney transplantation (KTx) is the modality of choice for both children and infants, in the latter group it is technically limited due to patient and vascular size, and as a consequence the majority of infants have to initiate either PD or HD. Both methods are feasible in infants, but PD is usually preferred. According to registry data PD served as the initial KRT therapy in 85–92% of infants in the USA and Europe [26, 15]. Advantages of PD include partial independence from the hospital setting, no need for vascular access, greater hemodynamic stability, better preservation of RKF, and fewer dietary and fluid restrictions. Drawbacks of PD are associated with burden of caregiver responsibility for conducting the treatment and requirement of a clean home with adequate storage space [27]. Contraindications for PD include conditions which compromise the abdominal cavity and peritoneum such as an obliterated peritoneal cavity, omphalocele or bladder extrophy. Hemodialysis is much less common in infants, although the use of chronic HD has been reported in children less than 10 kg [28]. Based on registry data, HD was the first dialysis modality in almost 14% of infants dialyzed in Europe and less than 3% in those from the USA [9, 10, 26]. Reluctance to utilize HD in small children is mainly due the need for long-term central venous catheters, which increases the risk of infectious and thrombotic complications. The latter may lead to damage to the central veins in patients who are potentially facing decades of KRT. An Italian study of infants receiving HD via CVCs

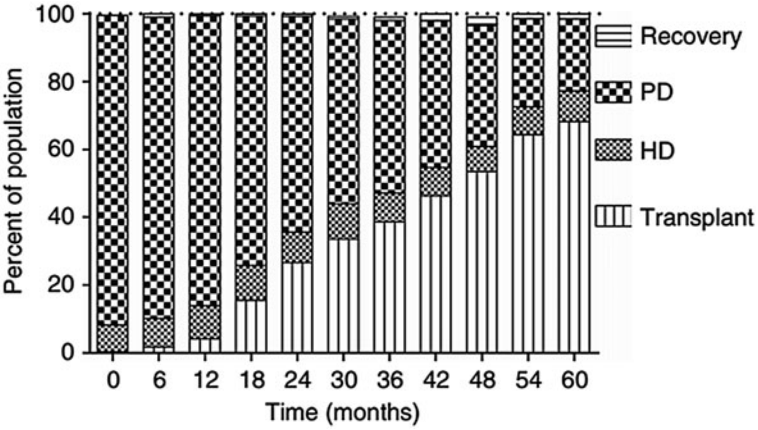

Fig. 1 Changes in treatment modality over time in infants who initiated dialysis in the neonatal period (adapted from Ref. [15] with modification)

confirmed CV thrombosis in 38% of patients [29]. Other important drawbacks include hemodynamic instability during hemodialysis sessions, blood loss, and exposure to blood products. The need for providing sufficient nutrition with fluid-based formulas in non-polyuric infants creates a problem of adequate ultrafiltration, which often necessities prolonged or frequent dialysis sessions. As a result of these disadvantages, it is usually only the infants with absolute contraindications for PD or patients with metabolic conditions in which HD gives better clearance, who initiate KRT with HD. The transition from one dialysis modality to the other is more frequent in infants compared to older children due to this high complication rate. In the ERA-EDTA report, one quarter of children between 0 and 4 years changed from PD to HD [30]. According the report of ESPN/ERA-EDTA, IPPN, Australian and New Zealand Dialysis and Transplant (ANZDATA), and Japanese RRT registries, 39.4% of the patients, who initiated KRT in the neonatal period, switched treatment modality during the first 2 years of life, most commonly due to KTx (Fig. 1) [15].

Peritoneal Dialysis

PD Catheters: Choice and Placement

Successful PD requires reliable access to the peritoneal cavity with a well-functioning dialysis catheter. Among the features potentially influencing catheter performance are catheter type, exit site orientation, placement technique, timing of first catheter use, and exit site care. Ideally, the placement technique, the characteristics of the PD catheter, and the care of the catheter should result in few mechanical and infectious complications. Generally infants are at increased risk of catheter related infectious and noninfectious complications. In a worldwide PD catheter outcome study, nearly 40% of infants required catheter revision, most commonly within 60 days of catheter placement [31]. The predisposition for young patients to experience access complications or failure may be related to a number of factors, including a lack of substantial abdominal musculature resulting in hernia formation, the use of diapers, and possibly the presence of immunodeficiency (such as low IgG levels), which may increase the likelihood of infection [31]. In order to decrease the rate of potential complications, a PD catheter should be placed by an experienced surgeon with expertise in infant catheter placement [32].

The first step in PD planning is catheter choice. There are various types of PD catheters available. The most widely used include single or double cuffed Tenckhoff catheters with either a straight or swan-neck subcutaneous portion and curled or straight intraperitoneal part. Whereas a double cuff catheter is usually preferred due to the ability to fix the external cuff in the subcutaneous tissue, in infants weighing less than 2–3 kg single cuff catheters can be used. It is also acceptable to place a single cuff vascular catheter in the peritoneal cavity if a Tenckhoff catheter is not available

[33, 34]. The length of the catheter has to be adjusted to patient size. The ideal length is measured by placing the internal cuff lateral and 1 cm above the umbilicus and then down to the symphysis pubis. In order to prevent contamination the exit site has to be positioned lateral or downward pointing and at a distance from any abdominal wall stoma or the diaper area [32].

When a PD catheter is inserted, a perioperative first-generation cephalosporin or gentamicin (refer to ISPD guidelines [32]) should be administered. The catheter can be placed either by open or laparoscopic procedure, with the former option being preferred in infants. A para-median or lateral entry into the peritoneum is performed with fixation of the internal cuff in the rectus muscle. If possible an omentectomy should be performed to prevent the omentum from clogging the side holes of the catheter. Closure of a patent processus vaginalis may be performed prior or at the time of PD catheter placement in order to prevent hernia formation. Immobilization of the catheter by dressing allows healing and prevents pulling and tearing. A delay of 2–3 weeks in dialysis following catheter implantation is beneficial, in order to prevent leaks and hernias. Fibrin glue at the internal cuff has been shown to decrease leaking complications [35]. As a gastrostomy button/tube is frequently needed to support nutrition, advanced surgical planning for PD catheter and gastrostomy placement is extremely important, and discussions between surgery and nephrology team members should address the optimal timing of both procedures. If possible, gastrostomy placement should either precede or be performed at the same time as the PD catheter insertion in order to reduce peritonitis risk [36–38]. If a gastrostomy has to be placed in a patient already on PD, an open procedure is necessary, and prophylaxis with antibiotic and antifungal therapy should be initiated. In such a case, it is advisable to hold dialysis for 2–3 days post-gastrostomy placement [32].

PD Prescription

There is no single optimal PD prescription, but the goals are to meet patient's ultrafiltration and solute clearance needs in order to optimize growth and development. Both continuous ambulatory peritoneal dialysis (CAPD) and automated peritoneal dialysis (APD) are feasible in infants. When first initiating, PD manual exchanges may be needed as fill volumes are likely to be very small; the initial fill volume of 10 ml/kg (absolute amount 20–30 ml per exchange in 2–3 kg infants), is lower than the minimum fill volume of 60 ml that makes up the “dead-space” of the tubing of cyclers. Fill volumes are increased gradually over days or weeks depending on the patient's tolerance. Once a fill volume of 80–100 ml is achieved, APD can be introduced. The target fill volumes in infants should not excide 600–800 ml/m^2, as higher values are associated with increased risk of hernia, leakage, and gastroesophageal reflux (GER) [21, 32, 36]. For these reasons the intraperitoneal pressure should not exceed 10 cm H_20 in this age-group. Low fill volumes result in a rapidly diminishing osmotic gradient and thus limited ultrafiltration; hence dwell times of 1 h or less are frequently utilized. Dwell times as short as 20 min may be used in neonates when rapid removal of small solutes is desired. In the setting of chronic PD, an initial empiric dwell time of 1 h is often used in infants, although consideration has to be made for clearance of molecules such as phosphorus which require longer dwell times. APD is the preferred modality in infants due to its flexibility and ease of making frequent adjustments in the rapidly growing patient. In order to preserve peritoneal membrane function it is advisable to use either fluids with the lowest possible glucose concentration that are “biocompatible,” with low glucose degradation products [39, 40, 5]. Calcium concentration (available as 1.25 or 1.75 mEq/l) may need to be individualized to provide sufficient calcium for growth and prevention of secondary hyperparathyroidism. Polyuric infants will usually require long dwell times to optimize solute clearances, while anuric patients need frequent exchanges and short dwell times to increase ultrafiltration.

Neonates and infants can experience excessive losses of protein via PD with studies demonstrating average daily losses of 250 mg of protein per kg of body weight or almost twice the peritoneal protein losses seen in older children. A dietary protein of at least 1.8 g/kg/day for the first 6 months of life is recommended, taking into account the dietary reference intakes (DRI) and peritoneal losses.

PD-Related Complications

Infant dialysis is associated with a high mortality and morbidity. This is influenced by a high incidence of multiple comorbid conditions, technical difficulties of infant dialysis, and high rates of both infectious and noninfectious complications [9, 15, 31]. The most common complication of PD continues to be peritonitis, with its incidence being much higher in infancy as compared older age groups [9, 11]. Catheter-related complications include mechanical problems such as catheter blockage or migration or PD fluid leakage such as with pleuroperitoneal or pleuropericardial fistulae, and exit site or tunnel infections. In the recent IPPN analysis, the overall catheter survival rate was significantly lower in infants compared with children in the older age groups [31]. In addition, the presence of any ostomy (including gastrostomy) is associated with a 55% higher risk for catheter failure [31]. As the underlying disease in most infants on dialysis is associated with tubular dysfunction causing polyuria and salt depletion (also aggravated by the loss of sodium in dialysate), hypotension, with its significant risks of cerebral or optic ischemia, is common in infants on PD [41]. For this reason many infants will require additional sodium supplementation. In contrast, chronic hypervolemia is common in anuric infants and may lead to hypertension and cardiac impairment [21].

Hemodialysis

Optimal hemodialysis (HD) in small babies is challenging and requires a thorough understanding of their unique age-related physiology. Many HD machines require double-lumen access with disproportionally large lines with a relatively large extracorporeal circuit, which necessitates blood or saline priming, thereby promoting HLA sensitization. The new machines designed for small babies (CARPEDIEM, NIDUS), which are currently being used in PICU for acute dialysis, allow successful delivery of continuous and intermittent diffusive blood purification modality to very small, using small CVLs, no blood primes, and excellent concordance between delivered and prescribed ultrafiltration. In future, these machines may be modified for maintenance dialysis in infants [42, 43].

HD Catheters

For chronic dialysis in infants soft blunt-tipped dual-lumen catheters are placed into the central veins. The preferred site is the right internal jugular vein, with the tip in the right atrium. Alternative sites are left internal jugular vein, subclavian, and femoral veins. Subclavian vein catheterization has been associated with a high risk of thrombosis, stenosis, and a shorter catheter survival, while femoral sites have a higher risk of exit site infections and bacteremia. The commercially available CVCs for neonates and infants include dual lumen 6 Fr catheters for neonates, 7 Fr for those weighting 3–6 kg and 8 Fr for infants >6 kg. If the body weight is <5 kg, single-lumen access may be more appropriate keeping in mind that the resistance of a tube, and therefore the blood flow, is inversely proportional to the fourth power of the radius (Poiseuille's law). Neonates can be dialyzed via the umbilical vein using a 5.0–8.5-Fr umbilical venous catheter. Two single lumen catheters placed into two veins (i.e., femoral veins) is possible. One catheter is advanced into the inferior vena cava ("venous" infusion), while the other is positioned in the internal iliac vein ("arterial" draw) [44].

HD Prescription

The HD prescription must be calculated and individualized for each patient to provide adequate solute clearance and fluid removal. Components of the HD prescription include the dialyzer, tubing (i.e., the HD circuit), blood flow rate, dialysate composition and flow rate, treatment duration and frequency, fluid removal goal, and anticoagulation. The dialyzer surface area should be equal to 75–100% of patient's body surface area in order to provide adequate fluid and solute removal and to minimize extracorporeal blood volume, which cannot exceed 10% of the patient's total blood volume (together dialyzer and tubing). As total blood volume is equal to 100 ml per kg in neonates and 80 ml/kg in infants, line priming with saline, albumin, or blood is usually needed. Effective membrane areas in dialyzers for infants range from 0.2 m^2, while neonatal lines allow priming volumes as low as 30 ml [45, 46]. The initial blood flow rate should not exceed 2–3 ml/kg/min, with a gradual increase to 5–8 ml/kg/min, depending on the patient's tolerance. The maximal ultrafiltration should not go beyond 5% of body weight per session or 1–2% of body weight per hour. In order to maintain good hemodynamic and nutritional status often long or frequent sessions are required [29]. The choice of dialysate should be individualized; in most centers bicarbonate is the standard buffer with its concentration varying between 32 and 40 mmol/L. Anticoagulation includes heparin, Low Molecular Weight Heparin (LMWH), or citrate depending on availability and center experience.

HD-Related Complications

The most common HD-related complications are associated with catheter performance, including poor blood flow due to malposition or thrombosis, and catheter infections. Six single center studies based on data of 9–11 infants report diverse catheter outcomes with median catheter survival ranging from 21 to 390 days and infection rate from 10 to 0.3 per 1000 CVC days [28, 47–50]. Central venous stenosis is a potentially life-limiting complication of chronic dialysis in children because it limits the efficiency and duration of peripheral access routes and reduces the number of vessels suitable for future catheterization and fistula placement. The risk of acute HD complications including intradialytic hypotension, air embolism, hemolysis, and acute anaphylactic reaction is similar to that in older age groups.

Growth and Nutrition

It is universally recognized that maintaining normal growth in infants on dialysis is a challenge for the multidisciplinary team. Some factors, such as the influences of comorbidities, prematurity, and low birth weight cannot be easily overcome. Other factors such as oligo-anuria, lung hypoplasia, episodes of sepsis and operative interventions aggravate feeding difficulties. However, the most important aspect to address in all infants on dialysis is nutritional intake. Nutrition is the predominant factor affecting growth in infancy, exceeding the influence from growth hormone during the first 2 years of life.

Anorexia and GER accompanied by recurrent vomiting are common. The rate of growth is greater than at any other time of life, being as high as 25 cm per year at birth, 18 cm per year at 6 months of age, and 12 cm per year at 12 months of age. It is not surprising therefore that without intensive nutritional management, loss of height standard deviation score (SDS) of as much as 0.6 SD per month can occur. This period without linear growth is very difficult to compensate [51].

]Many infants are unable to achieve adequate nutritional intake without resource to enteral feeding, although vomiting may mean that target formula volumes may not be achieved despite the use of a nasogastric (NG) tube. Vomiting may be due to a "full abdomen" from the peritoneal dialysate, constipation, and abnormal clearance of gastrointestinal hormones that control stomach emptying and GER. Medications such as prokinetic agents, proton pump inhibitors, and 5HT3 receptor antagonists may help with symptom reduction.

Concentrating infant formulas may reduce vomiting and provide the nutritional prescription in a smaller volume, e.g., increasing the formula concentration from 13% to 15% means that the volume can be reduced by 20 ml/kg/day. This is also important in the oliguric infant. Vomiting may also be reduced if feeds are given by slow continuous drip overnight with oral or bolus tube feedings offered during the day [52].

There are recent guidelines on the use of a gastrostomy tube in preference to an NG tube. In summary, a gastrostomy is less easily displaced; is hidden under clothing thereby removing the stigma of a chronically ill child; it is not associated with abnormal development of oromotor skills; can be safely used to feed overnight; and is associated with better growth, perhaps as a result of a decreased frequency of vomiting [5, 38].

Protein and Energy and Requirements

During PD, transperitoneal protein losses mean that the feed content of protein should be increased above the requirements for the normal population of the same age. These losses are increased further during peritonitis. Protein requirements for HD are lower than for PD patients, although there is amino acid loss in dialysate. The dietary aim is to enable normal growth and serum albumin, to maintain a urea level below 20 mmol/l and to control serum phosphate levels. Energy requirements do not differ from those for children with normal kidney function [53]. Further details on nutritional management are provided in ▶ Chap. 71, "Nutrition, Growth, and Development."

Salt and Water

Many infants on dialysis have CAKUT, and can continue with urinary salt and water losses for some time. In addition, losses into PD fluid are also important. Hypotension is common. Sodium supplementation and provision of adequate fluid intake in such infants seems counterintuitive, but chronic salt depletion is a well-known influence on growth [51].

CKD-MBD

Control of CKD-MBD is another challenge. The recommended daily calcium balance in the first year of life is 500–600 g, which is higher than at any other age, so that a baby on PD on a standard whey-based infant formula may be barely receiving adequate calcium, and rickets is common. Also what is frequently overlooked is that the normal range for plasma calcium is higher in the first year of life than at any other age [54].

Plasma calcium and phosphate can be manipulated by modifying dietary and dialysate intake, in addition to the use of phosphate binders and vitamin D [55]. The dialysate calcium can be increased from neutral (1.25 mmol/l) to higher levels of 1.75 mmol/L to allow diffusion of dialysate calcium into the blood. Like calcium, normal values of serum phosphate are also higher in infancy, but calcium containing phosphate binders are still often needed.

The normal population Reference Nutrient Intake (RNI) for vitamin D in the first year is 8.5 μg/day reducing to 7 μg/day at 7 months (340–280 IU). Standard whey-based infant formulas contain 1.2 μg (48 IU) vitamin D per 100 ml so babies will require a vitamin D supplement until they are on sufficient volumes of formula. However, it is likely that activated vitamin D will be necessary to control the PTH. Levels of PTH that are below two times the upper limit of normal have been correlated with better growth and less signs and symptoms of bone disease in older children but there is no specific data for infancy [55].

Further details on CKD-MBD management are provided in ▶ Chap. 72, "Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD)."

Long-Term Outcomes

Patient Survival and Causes of Death

Historically, infants who initiated chronic dialysis were less likely to survive or successfully receive a kidney transplant compared with the older children on dialysis [8]. However, in recent years (since 2000–2002), survival of infants on chronic dialysis has improved markedly [9, 11, 15, 56]. Combined data from the ESPN/ERA-EDTA registry, the IPPN registry, ANZDATA, and Japanese RRT registries indicated an estimated 2-year survival of 81%, and 5-year survival of 76% in neonates who initiated chronic dialysis during the period of 2000–2011 [15]. Data from the NAPRTCS registry reported that overall survival at 3 years after chronic PD initiation was 78.6% and 84.6% among the neonates and older infants (>1 month of age) who were treated in the period of 2000–2012, respectively [9]. Another analysis of the USRDS registry reported that patient survival at 1 and 5 years was 86.8% and 74.6% for those who initiated chronic PD as neonates and 89.6% and 79.3% for those who did so as older infants in the 2000–2014 era [11]. Nevertheless, patient survival remains lowest for infants on dialysis compared to older children on dialysis [8, 9]. The leading causes of death for infants on dialysis were infection (24.0–35.6%) and cardiopulmonary disease (16.7–23.5%) [10, 11, 15, 16]. This poorer survival in infants on dialysis is associated with the higher frequency of infection including peritonitis on PD, primary kidney diseases including autosomal recessive polycystic kidney disease, and the presence of comorbid conditions [27, 36, 56–58].

Kidney Transplantation

The primary goal for infants who initiate chronic dialysis is kidney transplantation. The ESPN/ERA-EDTA registry reports that 70.2% of infants had received a kidney transplantation within 5 years after the initiation of chronic dialysis therapy [10]. The USRDS registry reports that the median age at kidney transplantation was 2.0 years (IQR 1.5–2.9 years) and time to transplantation was 1.8 years (IQR 1.2–2.6 years) for infants who initiated chronic dialysis in the 2000–2014 era [11]. Furthermore, a report from combined data from the ESPN/ERA-EDTA registry, IPPN registry, ANZDATA, and Japanese KRT registries showed that at 5 years, of 264 children who initiated KRT in the neonatal period between 2000 and 2011, 99 patients had received a kidney transplant after a median of 25 (95 Confidence Interval 12.1–53.9) months, and 4 patients had received two kidney transplants [15].

Neurocognitive Outcomes

Because infancy is a sensitive time period for rapid neurodevelopment, infants requiring chronic dialysis therapy represent a very high-risk patient population for poor neurocognitive outcomes. Historically, before 1980, 20 (87%) of 23 infants with CKD developed profound neurological abnormalities including developmental delay, microcephaly, hypotonia, seizures, and dyskinesia [59]. Since the mid-1980s, long-term neurocognitive outcomes of infants requiring chronic dialysis have substantially improved, due to aggressive nutrition using nasogastric tube feeding, the elimination of aluminum as a phosphate binder, the provision of dialysis therapy and subsequent kidney transplantation [60]. However, recently two reports, in which patients with comorbidities including development delay and/or neurological disorder were excluded from the studies, indicated that long-term neurocognitive outcomes of infants who initiated chronic dialysis remains suboptimal [61, 62]. For example, one study reported that the mean (SD) patients' full scale intelligence quotient (FSIQ) scores were 78 (16.1) at an average age of 11.1 years, which were significantly lower than those of sibling controls (mean 94, SD 18.9; $p < 0.03$) [62]. Another study reported that mean (SD) FSIQ scores were 85 (20) at a mean age of 56 months, which was significantly lower when compared to normative data ($p < 0.015$) [61]. Noteworthy, both studies suggested that a shorter time on dialysis was associated with better neurocognitive outcomes [61, 62]. Given this result, a shorter dialysis vintage and earlier kidney transplantation may improve long-term neurocognitive outcomes in children who initiated chronic dialysis during infancy. Besides, further improvement of infant dialysis therapy is still needed to maximize the child's potential.

Conclusion

Chronic dialysis therapy is safe and feasible in infants. Mortality data have improved in children on dialysis over the past few decades, but the highest mortality rates are seen in infants who need chronic dialysis during the first year of life. PD remains the modality of choice, although improvements in dialysis technique, including the recent availability of dialysis machines designed for infants, makes HD a possible option.

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Adequacy of Dialysis

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Abstract

"Adequate" dialysis is often defined as a minimum clinically acceptable dose, below which occurs an unacceptable risk of poor outcomes. The term "dialysis adequacy" has been used for several decades primarily based on the measurement of small solute clearance including urea and creatinine and the findings from landmark adult-based clinical trials. Unfortunately, there are limited outcome-based data with respect to pediatric dialysis adequacy. Consequently, adult experience and the associated target clearances have been frequently extrapolated to pediatric patients, even though this point remains a matter of controversy. Small solute clearance represents only one part of the effectiveness of dialysis depuration, which should also include the clearance of larger and/or protein-bound molecules that

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play an important role in uremic toxicity and are more difficult to remove. Moreover, the dialysis prescription should be adjusted in order to maintain fluid balance and normal blood pressure. The achievement of dialysis adequacy should reflect measures that comprehensively aim to reduce child's morbidity and mortality and to maximize nutritional status, cardiovascular and bone health, growth, and patient/family health-related quality of life.

In this chapter, we discuss the tools that are available for estimating the dialysis dose delivered to pediatric patients treated with hemodialysis and peritoneal dialysis taking into account the specific metabolic, clinical, and rehabilitative needs related to differences of age, body size, residual renal function, nutritional status, and pubertal development. Formulae to estimate or calculate depuration targets are presented referring to pediatric metrics when available. Pediatric observational data related to dialysis adequacy as well as existing pediatric guidelines and recommendations are also reported.

Keywords

Hemodialysis · Peritoneal dialysis · Adequacy · Children · Solute removal · Urea kinetics · Total body water · Middle-sized molecules · Ultrafiltration · Residual renal function

Introduction

Adequacy of dialysis treatment was first analyzed in adult hemodialysis (HD) patients with studies focused on urea kinetics and small solute clearance. The resultant concept of "adequate" dialysis was created to define a minimum dialysis dose, below which a clinically unacceptable rate of negative outcomes might occur including patient hospitalization and mortality. The ability to define dialysis adequacy in children as associated with clinical outcomes has been hindered by the absence of large-scale, definitive outcome studies in pediatric HD and peritoneal dialysis (PD) populations due to issues including small sample sizes from pediatric centers and outcomes of interest that are more difficult to study (e.g., nutritional status, growth, cardiovascular health, health-related quality of life). Consequently, the extrapolation of the adult experience and the associated target clearances from guidelines to children became a matter of controversy.

Small solute clearance represents only one part of the effectiveness of dialysis depuration, which should also include the clearance of larger and/or protein-bound molecules that play an important role in uremic toxicity and are difficult to remove. Indeed, the achievement of dialysis adequacy should reflect measures that comprehensively aim to reduce child's morbidity and mortality and to maximize nutritional status, cardiovascular and bone health, growth, and patient/family health-related quality of life.

In this chapter, concepts surrounding dialysis adequacy and the process to achieve the abovementioned treatment targets in clinical practice will be discussed in the context of maintenance dialysis in the pediatric population.

Hemodialysis Adequacy

Overview

Since the landmark adult-based National Cooperative Dialysis Study (NCDS) conducted in the late 1970s, HD adequacy has been defined primarily by small solute clearance, and more specifically by the delivery of a minimum dose of urea clearance [1, 2]. Urea is an ideal representative marker of small solute clearance in HD due to its small molecular weight (60 Daltons), its ease of diffusion across extracorporeal dialysis membranes, and its relatively even distribution across total body water. Urea also represents a worthy nutrition marker as the amount of urea nitrogen generation in between HD sessions yields an estimate of dietary protein intake.

During the course of a HD session, there is an exponential decline of urea or blood urea nitrogen concentration [BUN] that follows first-order kinetics, which is a concentration-dependent process. In an intermittent HD schedule (e.g., thrice weekly), the [BUN] profile throughout the week follows a saw-tooth pattern as the post-dialysis [BUN] is typically reduced to a certain percentage of the pre-dialysis [BUN] and will then rise almost to the same level seen prior to the previous procedure. Early attempts to model adequacy from the NCDS were focused on the pre-dialysis [BUN] and the [BUN] time-averaged concentration (TAC) computed as the area under the saw-tooth curve divided by time [2]. Dialysis was thought to be "adequate" if the pre-dialysis or TAC BUN was appropriately low. However, low levels were also found to be associated with high mortality rates in select patients, likely from inadequate protein intake. Consequently, HD adequacy has evolved into metrics of urea removal utilizing pre and post dialysis [BUN] from a single HD session including urea reduction ratio, single pool Kt/V, equilibrated Kt/V, and standard Kt/V along with validated nutritional markers (normalized protein catabolic rate), all of which will be discussed in this chapter. Due to lack of both evidence and widespread use, machine estimated measures of HD adequacy including ionic dialysance and ultraviolet absorbance of spent dialysate will not be discussed.

Urea Removal Indices

Urea Reduction Ratio (URR)

The urea reduction ratio (URR) is a crude assessment of HD adequacy that can be obtained by noting the magnitude of the decrease in [BUN] during an HD session, usually expressed as a percentage of the pre-dialysis [BUN] [3]:

$URR = (C0 - C1)/C0$ *or* $URR = 1 - (C1/C0)$, where C0 is the pre-dialysis [BUN] and C1 is the post-dialysis [BUN] in mg/dL or mmol/L.

In order to reduce the risk of underestimating the post-dialysis [BUN] and overestimating the dose of dialysis due to access recirculation (AR), the post [BUN] sample should be obtained after slowing the blood flow to a rate at which AR is minimal (e.g., 50–100 ml/min) for approximately 15 seconds or by stopping the dialysate flow for three minutes before obtaining the sample [4].

In over 13,000 adult HD patients, URR <0.60 was independently associated with a higher risk of mortality, but URR has not been similarly studied in children [5]. The Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines recommend a minimum URR of 0.65 and a target URR of 0.70 to ensure one is consistently meeting the minimum threshold in 3×/week HD [4]. The main disadvantages of URR include not accounting for both volume contraction and the urea generated during an HD session.

Single-Pool Kt/V (spKt/V)

The term "single pool" assumes that dialysis is occurring in the context of a simple uniform urea space without consideration of disequilibrium between the patient's intracellular fluid (ICF) and extracellular fluid (ECF) compartments. Kt/V is a dimensionless ratio that represents the volume of blood cleared of urea ($K \times t$) divided by the urea distribution volume (V) during a single HD session. K is the dialyzer blood urea clearance (ml/min) at a specific dialysis blood pump flow rate, t is the duration of the HD session (min), and V is the distribution volume of urea (ml), which is close to total body water. Therefore, a Kt/V of 1 implies that the numerator (K x t), or the total volume of blood cleared of urea during HD, is equal to V. The fraction can be greater than 1 as the body water compartment can be dialyzed more than once during a single HD treatment. However, the amount of urea removed diminishes exponentially as the Kt/V is increased linearly. Figure 1 reveals the curvilinear relationship of URR and spKt/V.

The gold standard method of determining spKt/V is from urea kinetic modeling (UKM), an iterative mathematical model that requires specialized computer software or a programmable calculator (e.g., "Solute-Solver" at www.ureakinetics.org) and several variables from successive HD runs. Using pre- and post-dialysis [BUN] values, formal UKM solves for patient total body water (V, in mL) and interdialytic urea nitrogen generation (G, in mg/min). Values for V and G are then used to calculate Kt/V and the normalized protein catabolic rate (nPCR), which will be discussed later in the chapter.

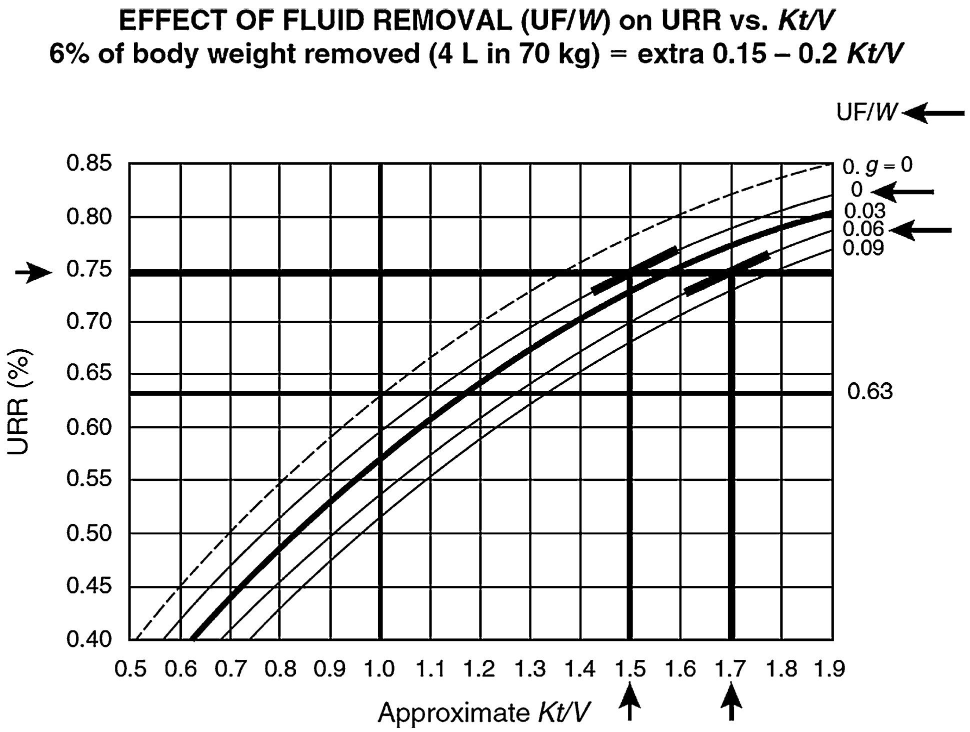

Fig. 1 Relationship of URR and Kt/V
Relationship between URR and Kt/V, taking into account urea generation and the effects of volume contraction (UF/W). Kt/V of 1.0 corresponds to URR (Urea reduction ratio) of 0.60 instead of 0.63 due to urea generation when there is no fluid removal. However, Kt/V of 1.0 can be attained with URR as low as 0.52 and average URR of 0.57 (heavy lined curve representing usual ultrafiltration of 3% of body weight). Similarly, URR of 75% can correspond to Kt/V of 1.5 in a patient with no fluid removal or to a Kt/V of 1.7 in patient from whom ultrafiltration is 6% of body weight. Reproduced with permission from Daugirdas JT. Handbook of Dialysis, fifth Edition. UF: Ultrafiltration (liters), W:Post dialysis body weight (kg), g: urea generation

As UKM is not routinely used in clinical practice, simpler spKt/V calculations are available based on its logarithmic relationship with the post- to pre-dialysis [BUN] ratio:

$spKt/V = -\ln(C1/C0)$ *or* $spKt/V = -\ln(1\text{-}URR)$, where ln is the natural logarithm, C1 is the post-dialysis [BUN], and C0 is the pre-dialysis [BUN] in mg/dL or mmol/L.

There are two variables that alter the relationship of URR and spKt/V not captured by the equation above. Figure 1 reveals that with increasing volume contraction represented from the different curves, a higher spKt/V is observed at any given level of URR. With fluid volume reduction, some urea is removed, but this removal is not associated with a change in urea concentration. Also when the small amount of urea generation that occurs during a dialysis session is factored into a standard 3.5 hour HD session, a Kt/V of 1.0 corresponds to a slightly lower URR of 0.60 instead of the expected 0.63 from the equation above. The Daugirdas II equation was developed to translate URR to spKt/V with adjustments for HD session length related urea generation and fractional volume removal [6]:

$spKt/V = -\ln(C1/C0 - 0.008 * t) + (4\text{–}3.5 * C1/C0) * 0.55\ UF/V$, where ln is the natural logarithm, C1 is post-dialysis [BUN], and C0 is pre-dialysis [BUN] in mg/dL or mmol/L, t is session length (hours), UF is volume of fluid removed during HD (liters), and V is the post-dialysis urea distribution volume (liters). V can be approximated as 0.55 x body weight (kg) or can be derived from pediatric anthropometric equations [7, 8], which will be further described in the PD adequacy section. The Daugirdas II formula has been shown to be a reliable alternative to formal UKM for spKt/V estimation in children of all sizes with <6% difference shown in paired data from 368 intermittent HD runs [9].

KDOQI recommends a minimum spKt/V of 1.2 and a target spKt/V of 1.4 for 3x/week HD [10]. A pediatric cross-sectional analysis from the United States (US) revealed that in 648 children receiving 3×/week HD, mean spKt/V was 1.6 with 89% achieving Kt/V $\geq$ 1.2 [11]. Observational data from US adolescents showed that older, larger, and African-American children are less likely to receive spKt/V $>$ 1.2 consistently [12].

Equilibrated Kt/V (eKt/V)

After dialysis is complete, urea diffuses back from the intra-cellular fluid (ICF) compartment of sequestered tissue sites to the patient's blood to cause a post-dialysis rebound phenomenon, which is largely complete by 30–60 min. Therefore, URR and spKt/V based on the typical sampling of [BUN] immediately after dialysis overestimates the true "equilibrated" clearance, known as eKt/V or double-pool Kt/V. Generally, eKt/V is approximately 0.2 Kt/V units lower than spKt/V in a standard 3–4 h HD session. However, the magnitude of urea rebound depends on the rate or intensity of HD given relative to body size and the degree of access recirculation. Four pediatric studies have revealed that the immediate post [BUN] rebounds by a mean of 17–25% to the equilibrated [BUN] at 50–60 min following HD [13, 14, 15, 16]. As it is impractical to have a patient wait for 1 h after HD to measure an equilibrated [BUN], many adult-based formulas have been devised to estimate eKt/V from spKt/V utilizing pre and post [BUN] obtained immediately after dialysis [17, 18, 19, 20].

A pediatric specific eKt/V equation has been validated by Goldstein and colleagues based on a logarithmic extrapolation of the rise of [BUN] in mg/dL from 30 sec to 15 min post HD to estimate the true equilibrated BUN (estBUN) [15, 16]:

$$estBUN = ([BUN_{15\,min} - BUN_{30\,sec}]/0.69) + BUN_{30\,sec}$$

The estBUN from this equation is then substituted for C1 in the Daugirdas II equation to obtain eKt/V. Compared to eKt/V from $[\mathrm{BUN}]_{50\mathrm{min}}$, this method has been shown to be the most accurate compared to other published eKt/V estimation methods utilizing $[\mathrm{BUN}]_{30\mathrm{sec}}$ [16]. However, the adult-based Daugirdas rate equation and Tattersall eKt/V equations utilizing $[\mathrm{BUN}]_{30\mathrm{sec}}$ also performed relatively well in children with a mean absolute difference of <5% from the true eKt/V [16]:

$eKt/V(Daugirdas) = spKt/V - 0.6 * (spKt/V)/t + 0.03$, where t is time in hours [20].

$eKt/V(Tattersall) = spKt/V \times t/(t + 35)$, where t is time in minutes [18].

Even though KDOQI has chosen not to recommend a minimum or target HD dose based on eKt/V, European best practice guidelines have set a minimum eKt/V threshold of 1.2 for 3×/week HD [21, 22]. A US-based study in children revealed that little difference in HD prescription management would result when comparing spKt/V versus estimated eKt/V values from the same HD treatment, justifying the use of spKt/V for adequacy assessments in children undergoing 3×/week HD [23].

Standard Kt/V (stdKt/V)

Increasing the frequency of HD alone increases dialysis efficiency while moving closer toward a more continuous pattern of clearance. The adequacy targets detailed above only apply to 3x/week HD, which has been the usual standard of care. With growing popularity of more frequent HD schedules, there has been a need to normalize and express the dose of dialysis independent of frequency and to reduce the delivered dose to a continuous equivalent weekly clearance. The stdKt/V concept suggests that therapies that achieve the same mean pre-treatment [BUN] are equivalent in delivered dose and therefore should achieve equivalent patient outcomes. This method was first developed by Gotch in 1998 and refined by Leypoldt in 2004 [24, 25]:

$StdKt/V = [168 * (1 - e^{-eKt/V})/t]/[(1 - e^{-eKt/V})/eKt/V)) + (168/(N * t)) - 1]$, where e is the exponential constant, t is time in hours, and N is the number of treatments/week.

eKt/V can be obtained with any of the known estimating equations utilizing spKt/V discussed previously, but none of these equations have been validated for therapies of arbitrary duration and frequency. Consequently, Leypoldt developed an equation to predict eKt/V for conventional HD, short daily HD, and daily hemofiltration therapy [25]:

$eKt/V = 0.924 * spKt/V - 0.395 * spKt/V/t + 0.056$, where t is time in hours.

The latest KDOQI update recommends for HD schedules other than thrice weekly, a target stdKt/V of 2.3 per week with a minimum delivered dose of 2.1 [26]. For pediatric 3x/week HD, Mammen and colleagues revealed that one should deliver a stdKt/V $\geq$ 2.0 in order to achieve spKt/V $\geq$ 1.2 and a stdKt/V $\geq$ 2.2 to ensure spKt/V $\geq$ 1.4 [27].

Issues of Scaling Dialysis Dose to "V"

As volume of distribution of urea (V) is closely tied to muscle mass, dosing dialysis by Kt/V may result in lower doses of dialysis for smaller patients, including women and children. Consequently, there has been some debate of whether dialysis dose should be scaled to body surface area versus V. In a study of children dialyzed 3x/week, a surface area normalized stdKt/V (SAN-stdKt/V) was significantly less in those younger than 10 years of age compared to adolescents [28]. With limited evidence and likely due to being a relatively new concept, KDOQI does not have any formal recommendations surrounding SAN-stdKt/V for adults or children at the current time.

Normalized Protein Catabolic Rate (nPCR)

Outside of small solute clearance, regular ongoing assessment of nutritional status is of utmost importance in an infant, child, or adult on dialysis. As traditional markers such as height, weight, and serum albumin are not always accurate indicators of nutritional status in children on maintenance HD, normalized protein catabolic rate (nPCR) has emerged as a useful and reliable tool. Normalized PCR, also referred to as the normalized protein equivalent of nitrogen appearance (nPNA), reflects the amount of protein in grams/kg/day that is catabolized in excess of the amount of protein that is synthesized. In a person who is in steady state (i.e., neither catabolic nor anabolic), the PCR reflects dietary protein intake. Therefore, an isolated nPCR can underestimate protein intake in a child with a significant growth spurt (anabolic state) and overestimate protein intake in a child on high doses of corticosteroids or suffering from a major infection (catabolic state). As it is difficult to directly measure protein intake, a method that is commonly used for estimation is to calculate the rate of increase in [BUN] between two hemodialysis treatments, otherwise known as G (mg/min). This can be obtained by formal UKM or can be reliably estimated from the difference of the pre and post [BUN] [29]:

$estG\ (mg/min) = [(C2 * V2) - (C1 * V1)]/t$, where C1 is post dialysis [BUN] in mg/dL, C2 is the pre dialysis [BUN] in mg/dL from the same session, V1 and V2 are the pre- and post-dialysis total body water values in dL respectively, and t is the interdialytic time in minutes.

Then nPCR can be calculated using the modified Borah equation [30]:

$nPCR\ (grams/kg/day) = 5.43 * estG/V1 + 0.17$, where V1 is the post-dialysis total body water as above.

Pediatric studies have demonstrated that in comparison to serum albumin, nPCR is a valuable nutrition marker in malnourished patients receiving intradialytic parenteral nutrition (IDPN), and that nPCR <1 gram/kg/day was predictive of persistent weight loss in adolescents [31–33]. Another study in children showed nPCR is overestimated slightly when using pre and post [BUN] from the same HD session compared to using the pre [BUN] from the subsequent HD session [34].

Residual Kidney Clearance

Although sometimes difficult to collect in children, regular assessment of residual urea clearance (Kru) through a timed hour urine collection in between HD sessions can be important to determine if the patient is meeting combined dialysis and residual kidney adequacy targets and is on the appropriate HD prescription. The Kru calculation is as follows:

Kru = ([UUN]/[BUN]) * urine flow rate (ml/min), where UUN is the urine urea nitrogen concentration and BUN is blood serum urea nitrogen concentration, often estimated from the average of pre- and post-dialysis [BUN] if the collection occurs during the entire interdialytic period. If the urine collection is 24 hours prior to dialysis in 3x/week HD, one can assume that the average [BUN] concentration during the collection is approximately 90% of the pre-dialysis [BUN].

The minimally adequate HD dose recommended by KDOQI is often given in the context of those with Kru greater than or less than 2 ml/min/1.73m^2. For those with Kru $\geq$2 ml/min/1.73m^2, the dose of dialysis may be reduced provided Kru is measured regularly to avoid inadequate dialysis, but this practice has not been adopted regularly by pediatric care providers. Kru can be combined with dialyzer urea clearance by converting it to a weekly clearance (Kru x 10,080/V) and adding it directly to stdKt/V. Daugirdas has also developed a stdKt/V equation that includes effects of fluid removal and Kru [35]. Kru of 3 ml/min is equivalent to a stdKt/V of approximately 1.0 per week. Inclusion of Kru is also advantageous because it allows for a more accurate measurement of G and nPCR, which are otherwise underestimated in patients with significant residual function. For an accurate nPCR, total urea nitrogen lost in the urine must be accounted for during the interdialytic period. With this measurement, the following term can be added to the calculated nPCR [36]:

> ([UUN] * 150) / (interdialytic interval (hours) × weight (kg)), where UUN is urine urea nitrogen in grams.

Is More HD Better?

Unfortunately, there are limited outcome-based data on pediatric HD adequacy due to several epidemiological barriers. Consequently, KDOQI recommendations surrounding pediatric HD adequacy including minimum delivered dialysis doses are mostly based on extrapolations from adult based studies. However, a pediatric section of the guidelines suggest that adequacy assessments should occur monthly and that children should receive at least the delivered HD dose as recommended for the adult population [4]. This is supported by an analysis of 613 US adolescents on 3x/week HD revealing spKt/V $<$ 1.2 was independently associated with a higher risk of hospitalization [37].

The landmark HEMO randomized study in adults dialyzed 3x/week found that increasing delivered doses higher than KDIGO recommended thresholds did not improve outcomes including mortality and hospitalization [38]. Whether these findings are generalizable to infants, children, and adolescents on HD is questionable considering that the relationship of dialysis dose to other important pediatric outcomes including growth, development, and quality of life is not well known. KDOQI states that considerations should be made to prescribe higher dialysis doses along with higher protein intakes in children who have greater nutritional needs to support growth and development [26]. This is based on pediatric observational data that suggests “more is better.” Tom and colleagues showed that growth was optimized with a target spKt/V $>$ 1.8 in 3×/week HD (5 h/session) and a high protein intake, even without the effects of growth hormone [39]. Equilibrated Kt/V $>$ 1.4 was also shown to be associated with improvements in inflammation status in 3x/week HD [40, 41].

More frequent HD and use of hemodiafiltration (HDF) has also been associated with improved outcomes in children. Fischbach and colleagues showed improved growth with HDF (5–6 sessions per week) and spKt/V $>$ 1.5 [42]. Goldstein and colleagues revealed that when switching from 3 to 6×/week HD, all children with nPCR $<$1 g/kg/day increased to $>$1.1 g/kg/day with stdKt/V $>$ 2.0 achieved in 85% of assessments [43]. However, the benefits seen in more frequent HD and HDF may not be related to small solute clearance and may be related to improved clearance of middle molecules, phosphate removal, as well as tighter fluid control leading to improved blood pressure and cardiovascular health. This is supported by a recent multicenter international study showing that despite similar Kt/V values, improvements in growth, blood pressure, interdialytic weight gain, and other cardiovascular measures were seen in children on HDF versus conventional hemodialysis [44]. This example nicely shows that although meeting adequacy targets for small solute clearance is important, several other domains of patient care adequacy are equally important for optimizing the care and quality of life of our maintenance HD patients.

Peritoneal Dialysis Adequacy

Overview

During the 1990s, the influence of small solute clearance on patient outcomes on peritoneal dialysis (PD) was a major focus of interest. Observational studies in adults on continuous ambulatory peritoneal dialysis (CAPD) suggested that improved patient survival and lower morbidity was associated with higher clearances of both urea and creatinine [45, 46]. As a consequence, small solute clearance was considered the key criterion of PD adequacy in clinical practice guidelines such as KDOQI [47]. Subsequently, a re-analysis of the landmark CANUSA study along with two other randomized controlled trials, including the ADEMEX study and another study from Hong Kong [46, 48, 49, 50], were unable to demonstrate any clear survival advantage with increases in peritoneal small solute clearance and showed that residual renal function (RRF) is a much stronger predictor of patient survival than urea and creatinine peritoneal clearance. Possible explanations for failure of increased PD dose to improve outcomes include higher intraperitoneal pressure associated with larger exchange volume, failure to increase clearance of middle molecules, and the consequences of increased exposure to glucose-based dialysate [51]. Moreover, the effect of the total fluid removal on mortality was not evaluated.

In children, even more so than in adults, adequacy of PD treatment cannot be exclusively defined by targets of solute and fluid removal [52]. Clinical assessment of adequacy of PD treatment should comprehensively take into consideration a series of clinical, metabolic and psychosocial aspects such as: electrolyte and acid-base balance; calcium and phosphate homeostasis; control of anemia; hydration status and blood pressure control; nutritional status and dietary intake of energy, proteins, salts, and trace elements; growth; psychomotor and mental development; level of psychosocial rehabilitation. Some of these issues which are more directly related to the delivered PD dose will be addressed in this part of the chapter.

Small Solute Clearance

In the absence of definitive outcome data in pediatrics to indicate that any measure of dialysis adequacy is predictive of well-being, morbidity, or mortality, 2006 KDOQI guidelines [53] stated that the target “delivered” small solute clearance in children should meet or exceed adult standards. These guidelines recommended the exclusive use of urea removal scaled for urea distribution volume (Kt/V_{urea}) for the evaluation of small solute clearance, with a total (peritoneal plus RRF) minimum target of Kt/V_{urea} of at least 1.7 per week. This recommendation was confirmed by pediatric PD guidelines published in 2020 [54]. Prior KDOQI guidelines [47, 55] also gave an opinion based recommendation for a minimum target of weekly creatinine clearance (CrCl) (Table 1) of 60, 63, and 66 Liters/1.73 m^2 for adults on CAPD, continuous cycling PD (CCPD), and nightly intermittent PD (NIPD) respectively, that took into account the continuous or intermittent regime of the three PD modalities, and were incorporated in pediatric European guidelines [56].

For practical reasons, peritoneal and RRF are usually added to determine total clearance, even if they may have a different impact on patient outcome. Some of the issues surrounding the evaluation of residual kidney clearance were addressed earlier in the HD adequacy section of this chapter. The term “delivered” refers to the actual dose the patient is receiving based on direct measurement of solute clearances and fluid removal, and not to an estimated value obtained by using a kinetic

Table 1 Peritoneal dialysis adequacy related formulae in children

Anthropometric total body water (TBW) prediction equations $^{(8)}$ Boys: $\text{TBW} = 0.10 \times (\text{Ht}*\text{Wt})^{0.68} - 0.37 \times \text{Wt}$ Girls: $\text{TBW} = 0.14 \times (\text{Ht}*\text{Wt})^{0.65} - 0.35 \times \text{Wt}$ Ht = height, cm; Wt = weight, kg
Body surface area (BSA) $^{\lceil}$ [60]$^{\rceil}$ $\text{BSA}\ (\text{m}^2) = 0.0235 \times \text{height (cm)}^{0.42246} \times \text{weight (kg)}^{0.51456}$
Protein nitrogen appearance (PNA) $^{(55,\ 61)}$ PNA (g/kg/day) = [(6.49 × UNA) + (0.294 × V) + protein loss (g/24-h)] / weight (kg) UNA = urea nitrogen appearance, resulting from 24-h urinary urea nitrogen (g) + 24- h dialysate urea nitrogen (g); V = urea distribution volume (L)
Kt/Vurea (per week) Kt/Vp (peritoneal) = (volume of dialysate (L 24 h) x DUN dialysate/ BUN plasma) × 7 / TBW (L) Kt/Vr (residual) = (volume of urine (L 24 h) × UUN urine/BUN plasma) × 7 / TBW (L) Kt/Vt (total) = Kt/Vp + Kt/Vr
Creatinine clearance (CrCl) (per week) CrClp (peritoneal) (L/week/1.73 m^2) = (24-hour D/P Cr x 24-hour dialysate volume in L) x 7 × 1.73 m^2 / BSA (m^2)

**CrClr (residual)(L/week/1.73 m^2) = 0.5 × [(24-h urine volume in L x urine Cr concentration /1440 min/day x serum Cr concentration) + (24-h urine volume in L x urine BUN concentration / 1440 min/day x serum Cr concentration)] × 1.73 m^2/BSA (m^2) × 7

CrClt (total) = CrClp + CrClr

**For peritoneal dialysis adequacy purposes, renal "CrCl" is the average of the urinary creatinine and urea clearances.

g = grams, h = hours, L = liters; BUN = blood urea nitrogen in mg/dL or mmol/L; DUN = dialysate urea nitrogen in mg/dL or mmol/L; UUN = urine urea nitrogen in mg/dL or mmol/L; D/P = dialysate to plasma solute concentration; Cr = creatinine in mg/dL or umol/L.

modeling program that are useful tools to improve the personalization of PD prescription, especially in APD patients [57, 58]. Assessment of the delivered PD dose is based on the measurement of dialytic and kidney clearance obtained through 24-hour dialysate and urine collection. All dialysate effluent produced for 24 hours should be collected, including daytime exchanges if performed. Total volume should be measured, and a small sample gathered after mixing the collected fluid thoroughly. Table 1 shows the formulae and calculation of weekly $\text{Kt/V}_{\text{urea}}$ and CrCl used for PD adequacy based on dialysate and blood samples. Compared to CAPD, NIPD patients may demonstrate variability in serum levels of urea depending on the time of the day of blood collection. When blood samples are obtained in the morning soon after completing a cycle of NIPD, dialysis adequacy as measured by Kt/V may be overestimated. Consequently, a mid-afternoon blood urea sample in NIPD may potentially be more accurate for determination of adequacy, even if difficult to obtain for practical reasons.

Solute clearance should be measured within the first month after initiating PD when full fill volume, scaled to patient's body surface area (BSA in m^2), and stable PD prescription have been achieved; thereafter, it should be measured at least once every six months if the patient is clinically well. More frequent measurements should be conducted when dialysis clearance may have been compromised (e.g., after repeated peritonitis episodes), when there has been a rapid decrease of RRF, or there is clinical suspicion of inadequate dialysis such as increased fatigue, decreased appetite, worsening fluid retention, and/or difficult to control hypertension. Moreover, if a patient is not doing well and has no other identifiable cause outside of uremia, a trial of increased dialysis is indicated.

Historically, both $\text{Kt/V}_{\text{urea}}$ and creatinine clearance (CrCl) have been employed to evaluate PD clearance. A discrepancy between urea and creatinine PD adequacy parameters has frequently been observed in adults and in children on APD [56], since urea clearance is more related to the total dialysate volume and the number of exchanges, while CrCl is predominantly affected by the dwell time (i.e., the duration of peritoneal membrane (PM) contact with dialysis fluid, also called "contact time") and by RRF. The finding of adequate Kt/V_{urea} values associated with inadequate CrCl values can be related to a hyperpermeable PM state or an insufficient intraperitoneal volume, since both are associated with a greater removal of urea than creatinine [59]. Finally, scaling of Kt/V_{urea} to body weight (BW) and CrCl to BSA may influence values obtained in infants and small children as a result of a higher BSA/BW ratio [56]. More recent recommendations suggest the determination of Kt/V_{urea} alone without CrCl for PD patients based upon the simplicity of its calculation, and because studies on adult PD patients did not provide evidence of a benefit in terms of patient outcome when expressing clearance in any manner other than Kt/V_{urea} [53, 54].

Kt/V_{urea} is normalized for urea distribution volume (V), which is assumed to equal total body water (TBW). Therefore, accurate estimation of TBW is a critical component of dialysis dose measurement. Since gold-standard isotope dilution techniques to determine TBW are laborious, costly, and not widely available, anthropometric prediction equations based on height and weight are commonly used to estimate TBW. Equations derived from healthy children [7] systematically overestimate TBW in pediatric patients receiving PD, and a new set of anthropometric TBW prediction equations have been developed in this patient population, and validated by comparison with the determination of TBW by means of heavy water (H_2O^{18} or D_2O) dilution technique [8]. These formulae are based on a new anthropometric parameter called *height times weight* that correlates linearly with TBW when both values are log-transformed and are reported in Table 1.

In conclusion, numerical targets of small solute clearance, as defined by currently available guidelines, should be interpreted cautiously and in the context of a thorough and repeated assessment of patient clinical and biochemical parameters. Whether the target clearances advocated for adults should serve as the standard for children remains an issue where evidence is scant.

Clearance of Middle-Sized Molecules and Phosphate

Failure to achieve adequate clearance of middle-sized molecules (300–5000 Daltons) and of phosphate is one possible explanation for the lacking effect of increased dialysis dose on improving patient survival [51]. In children, Shroff and colleagues found a correlation between serum phosphate levels and carotid intima-media thickness, a surrogate marker of cardiovascular disease [62]. Small solute and middle molecules clearances respond differently to changes in PD prescription. While the former is mainly determined by the frequency of exchanges and total dialysate volume, the latter depends more on the contact time between dialysate and the peritoneal membrane. Middle-sized molecules and phosphate removal is slow and equilibration with plasma levels only occurs with a long dwell such as the night dwell in CAPD or the daytime dwell in CCPD [63]. Removal of middle-sized molecules and low-molecular weight proteins, such as β_2-microglobulin and leptin, mainly depends on RRF [64, 65]. Moreover, an increasing restriction coefficient was reported for macromolecules with time on chronic PD, which is associated with increased size selectivity and a reduced peritoneal permeability for higher molecular weight solutes [66]. Hence, particular attention should be paid to middle-sized molecule clearance, especially in children on nightly intermittent PD, and as RRF declines. In order to increase the removal of middle molecules, a continuous PD regimen (CAPD or CCPD) can be adopted. Increased β_2-microglobulin and leptin clearance have been reported in patients receiving a long dwell with icodextrin solution [67].

The efficacy of phosphate elimination by a standard APD regimen is limited and independently predicted by total fluid turnover, the number of cycles, 2-hour dialysate-to-plasma (D/P) phosphate, dwell time, and ultrafiltration. It should be considered that while performing a peritoneal equilibration test (PET), the time needed to obtain a D/P for phosphate of 0.50–0.60 is three to four times longer than for urea. Schmitt and colleagues [68] raised the issue whether dialytic phosphate removal might provide a more reliable measure of dialysis efficacy than urea and creatinine clearance. By studying peritoneal phosphate kinetics and daily dialytic and renal phosphate elimination in children receiving APD, they found that 4-hour D/P phosphate and 24-hour phosphate clearance were lower in hyperphosphatemic children (3.38 +/− 1.17 vs 4.56 +/− 1.99 L/1.73 m^2/day, $p < 0.05$). Peritoneal transport state defined by the creatinine equilibration pattern poorly predicts daily phosphate clearances, suggesting that a specific evaluation of the D/P phosphate ratio could be done to define an individual's phosphate transport category. As overall PD phosphate clearance is limited, dietary counseling for phosphate intake restriction and phosphate binders use are needed to achieve the target of normal serum phosphate level that is associated with decreased risk of cardiovascular disease and better control of bone health [54].

Fluid Balance and Ultrafiltration

The PD prescription should be adjusted continuously to maintain fluid and sodium balance and normal blood pressure (BP). The North American Pediatric Renal Trials and Collaborative Studies [69] showed that 57% of nearly 4000 patients on dialysis had blood pressure values above the age-, sex-, and height-specific 95th percentile. The European Pediatric Renal Replacement Therapy Registry [70] reported systolic or diastolic BP standard deviation score (SDS) > 95th percentile irrespective of the use of medication in 35.5% of 851 pediatric PD patients. The International Pediatric Peritoneal Dialysis Network (IPPN) reported an overall prevalence of left ventricular hypertrophy (LVH) of 48.1% among 507 pediatric PD patients treated in 55 centers around the world and found that high total fluid output (urine plus PD-related UF) was protective from concentric LVH, especially in APD patients [71].

Routine evaluation of volume and sodium status, daily UF volume, and 24-hour urine volume is essential in the process of attaining adequate PD. A patient's extracellular volume is not easily measured and frequent assessment of patient "target weight" or "dry weight" mainly relies on clinical judgment. In clinical practice, the desirable target weight of a PD patient could be reasonably approximated to the weight at which the patient is free of edema and normotensive with minimal need for anti-hypertensive medications. Since fluctuations in patient weight secondary to growth and to changes in nutritional status may occur, reevaluation of target weight at each clinic visit is mandatory [54]. In addition to physical examination for edema, BP measurement and laboratory parameters estimating hemodilution (hemoglobin/hematocrit; serum albumin levels), volume assessment by means of bioimpedance spectroscopy has been proposed in children on dialysis [72]. Other volume assessment tools exist, such as measurement of circulating natriuretic peptide, lung ultrasound, inferior vena cava diameter, and collapsing index, but data on their use in children are limited.

Peritoneal membrane transport characteristics affect net fluid removal at a given dwell time by determining the osmotic gradient time curve of each individual patient. Patients with high or high average peritoneal transport are more at risk of developing fluid overload. A modification of the standard PET utilizing 4.25% dextrose solution can be employed to better evaluate UF kinetics and the maximum dip in D/P sodium, which reflects sodium-free water transport. If the patient has a high transport status, improved UF will be obtained by increasing fill volume as tolerated and/or shortening dwell time. In patients with decreased sodium-free water transport and no dip in D/P sodium after 1–2 h of the dwell, there will be no benefit from the use of a high dialysate glucose concentration. La Milia and colleagues [73] suggested calculation of the exact volume of free water transport by measuring the amount of sodium transported through the small pores over an 1-h dwell; since the total UF volume is known, subtracting the small pore transport from the total transport will give the amount of water transported through the aquaporin channels.

Any efforts should be maximized to employ the lowest possible glucose concentration required to achieve the desired UF rate to minimize glucose related toxicity. Further interventions to maintain patient fluid balance include:

- Dietary counseling on sodium and fluid restriction taking into account renal and/or dialysis-related sodium losses, since sodium depletion may result in hypotension and impaired growth, especially in infants.
- In children with RRF, use of loop diuretics and renin-angiotensin aldosterone system (RAAS) inhibitors (in patients who require antihypertensive medications, and with strict monitoring of potassium serum levels) [54].
- Evaluation of peritoneal transport characteristics by means of PET with subsequent adaptation of fill volume, dwell time, and PD solution tonicity.
- Use of icodextrin solution during a single prolonged dwell (daytime dwell in children on CCPD) with a fill volume above 550 ml/m^2 BSA seems to facilitate UF [74]; UF can be suboptimal in younger children due to a higher rate of icodextrin reabsorption [75].

- Adapted PD [76], combining short and long cycles in the same APD session, to optimize sodium and fluid removal (see PD chapter).

Clinical Correlates of PD Adequacy

Large-scale, Large scale, prospective studies in children on PD are lacking children are lacking owing to the small number of patients per center, and the relatively short period of time on dialysis prior to renal transplantation. Nevertheless, some pediatric observational studies effectively analyzed the relationship between PD dose and selected clinical aspects including growth and nutrition.

Growth is a potentially valuable outcome measure specific to pediatrics. Multivariable analysis from a multicenter study [57] showed a weak positive correlation of height standard deviation score (SDS) with dialytic creatinine clearance, and a negative correlation with peritoneal transport status. Children with a high transporter status were at increased risk of poor growth, which is difficult to explain. Growth rate is possibly more related to residual kidney solute clearance than to PD efficacy [77]. An IPPN study showed that in children treated with more biocompatible PD solutions with neutral pH and low glucose degradation products (GDP) content, growth was significantly better than in children using conventional PD fluids [78]. These data suggest that reducing inflammatory processes and carbonyl stress accomplished with reduced GDP exposure might improve growth rate.

In children on PD, dietary protein intake is only inconsistently correlated with delivered Kt/V_{urea} [79, 80, 81]. The relationship between Kt/V_{urea} and the protein equivalent of nitrogen appearance (PNA) calculated by means of the modified Borah equation utilizing timed dialysate and urine collection (Table 1) has often been criticized as merely being the result of mathematical coupling [61]. Finally, a higher Kt/V_{urea} was associated with a lower serum albumin level in children, suggesting that enhancing PD dose (i.e., to a $Kt/V_{urea} > 2.75$) may provide no further benefit, potentially owing to an increased loss of albumin into the dialysate [82]. However, in patients with high peritoneal transport and high $Kt/V_{urea,}$ serum albumin levels may be spuriously low in the presence of fluid overload due to poor UF and hemodilution effects.

The Role of Residual Renal Function

Prospective randomized trials of dialysis adequacy and observational studies in adult patients have confirmed that RRF is a much stronger predictor of patient survival than peritoneal clearance [48, 49, 50]. No data from large-scale trials on the relationship between RRF and patient outcome are available for children. However, an observational study reported higher growth velocity in children on PD with RRF compared to anuric children despite similar total solute clearance rates in both groups [77]. Other pediatric studies show that LVH is associated with a daily urine output of less than 0.5 L/m^2/day [71], and loss of RRF predicts diastolic dysfunction [83].

The rate of RRF decline in pediatric patients on PD was reported to be slower than in patients on HD, and high urine volume at the initiation of PD is predictive of sustained diuresis [84, 85]. Avoidance of excessive UF rates and of intradialytic hypotension, principally during HD, but also during PD sessions is recommended [86]. Studies of adult PD patients showed that low GDP fluids have the potential to better preserve RRF [87, 88, 89, 90]. However, multivariate analysis on 181 PD children attributed only a marginal increase of daily urine volume to the use of biocompatible solutions [86].

Preservation of RRF also includes avoiding nephrotoxic insults, such as exposure to nephrotoxic medications and radiocontrast agents, prompt resolution of volume depletion episodes, and prevention and early treatment of urinary tract infection. In PD patients, particular attention, including surveillance of serum drug levels, is required when aminoglycoside antibiotics are used to treat peritonitis in view of their nephro-, oto-, and vestibular toxicity, especially when repeated antibiotic course are required. Diuretic therapy can be used to increase urinary water and salt excretion and to delay the occurrence of oligoanuria [86]. Data from adult PD patients support the use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) to slow RRF decline [91, 92]. Experience in pediatric PD patients is still limited. Data from the IPPN registry showed that the use of RAAS inhibitors tended to increase the risk of developing oligoanuria in a cohort of 401 incident PD pediatric patients [86]. In summary, interventions that may contribute to the preservation of RRF should be adopted whenever possible. RRF should be measured routinely by 24-h urine collection (Table 1) and PD prescription should be adjusted to its decline in a timely fashion, in order to prevent inadequate treatment.

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Renal Transplantation: Evaluation of Children and Donors

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Abstract

Kidney transplantation is considered a definitive modality of kidney replacement therapy. Living-related and preemptive renal transplantation is one of the crucial factors allowing planned and rapid access to optimal treatment. Given the worldwide shortage of kidney donors, the criteria for selecting suitable deceased donor kidneys must be carefully weighed up against the recipient's needs. Significant progress in immunologic testing and monitoring, as well as careful pretransplant evaluation of recipients and donors has improved the long-term outcomes of pediatric renal transplantation.

Keywords

Kidney replacement therapy · Living-related kidney transplantation · Deceased-donor kidney transplantation · Recipient and donor evaluation · HLA matching · Donor-specific antibodies (DSA) · Graft survival · Graft nephrectomy

Introduction

Kidney transplantation in children is a relatively new field. While the first successful kidney transplants were carried out more than 50 years ago, renal transplantation in children in the 1970s was still rare and risky, and only with improved surgical techniques and better immunosuppression did numbers and outcomes begin to improve in the 1980s. Further improvements in graft survival and patient survival have occurred since 2000, possibly due to improved immunosuppression with the greater use of tacrolimus and mycophenolate, but this has plateaued more recently [1].

Many of the issues with kidney transplantation for children are the same as for adults, but concerns around graft quality, longevity, risk of infection, cancer, access to transplantation, and disease recurrence differ between these age groups.

As compared to adults with end-stage kidney disease (ESKD), most children are eligible for a kidney transplant, and in various jurisdictions receive priority for deceased donor organs. In the majority of countries, parents are a major source of kidney donation, with mothers being kidney donors more often than fathers, then extended family, and occasionally, altruistic donors. There has been an increasing use of deceased donor organs, and the vast majority of these are adult donors, although there is a growing desire to address the use of younger and even neonatal donors. Access to rapid and high-quality donation is of major importance for the lifetime outcomes of children with kidney failure. Full informed consent must be obtained from all donors, meeting recommendations of the Declaration of Istanbul [2, 3]. The pretransplant evaluation of children and donors is central to improved outcomes. In this chapter we discuss the workup of the recipient and of living and deceased donors.

Recipients

Renal transplantation is required in children with stage 5 chronic kidney disease (CKD), when glomerular filtration rate is maintained below 15 mL/min/1.73 m^2 and complications related to CKD become clinically overt. There can be a marked variation in uremic symptoms that patients show at different levels of glomerular filtration rate (GFR): some children demonstrate severe uremic symptoms even at GFR > 15 mL/min/1.73 m^2, while others remain asymptomatic even at much lower GFRs. The severity of clinical symptoms, like intractable hypertension, chronic fluid excess, metabolic derangements, acidosis, fatigue, poor energy levels and physical activity, and growth retardation must be considered in the decision to proceed with transplantation. The development of a pathway and checklist for potential donors and recipients will facilitate preemptive transplantation or placing the child on a deceased donor waiting list at the earliest opportunity [4, 5].

There is no universal age and body weight limit for recipients; however, renal transplantation in children below 10 kg is not common. Anatomical problems such as vessel diameter incompatibility, insufficient space in the retro/intraperitoneal area, and risk of renal graft hypoperfusion and thrombosis are major problems limiting the use of renal grafts from much older donors in minor recipients. The results of renal transplantation in children between 8 and 10 kg of body weight are, however, not inferior to older recipients [6, 7], but only performed in selected centers with relevant experience.

There are three distinct baseline clinical settings for renal transplantation. It may be performed before introduction of chronic dialysis, as a primary, preemptive form of kidney replacement therapy (KRT); in patients undergoing chronic dialysis; and as retransplantation in patients who have lost the function of a previous renal graft. In the first two settings, several aspects of the primary underlying kidney disease must be carefully evaluated in terms of the potential to modify management during or after transplantation. Patients with glomerular disease more often receive a transplant after a period of dialysis therapy, while children with nonglomerular disease more often receive a preemptive transplant. In cases of retransplantation, the clinical course of primary transplantation and the cause(s) of graft loss are more important for the outcome of the subsequent transplant [8].

Primary Disease and Renal Transplantation

One of the core assessments of candidates for renal transplantation is the risk to transplant outcome due to the primary disease. Congenital anomalies of the kidney and urinary tract (CAKUT) are the major causes of CKD in children. Lower urinary tract abnormalities can adversely affect the transplanted kidney, whereas isolated renal hypodysplasia has no real impact on transplant function. Glomerular diseases have the potential to recur after transplantation and persistent chronic glomerular injury frequently causes native proteinuria even in late CKD, as well as volume-independent hypertension. Ciliopathies affect surgical management due to the high urine output of native kidneys (as in polycystic kidney disease, in children, mainly recessive (ARPKD)) and may cause liver failure in some. Thrombotic microangiopathies (TMA) such as atypical hemolytic uremic syndrome (aHUS) may recur after transplantation and require disease-specific posttransplant management (long-term eculizumab prophylaxis or treatment). Metabolic diseases (like primary hyperoxaluria type 1) may cause severe extrarenal abnormalities and therefore may be an indication for liver-kidney transplantation, either as combined or sequential transplants. In all relevant cases, genetic testing should be performed before transplantation, with special regard to genetically determined rare diseases, which have the potential to recur (such as TMA or some glomerular diseases), or are associated with risk of malignancy and warrant recipient nephrectomies (such as WT1), or may impact the selection of living-related donors.

Evaluation of Native Kidneys and Urinary Tract Before Transplantation

Imaging of the kidneys and urinary tract followed by a careful assessment of bladder function is required in children with an abnormal lower urinary tract such as those with CAKUT, bladder extrophy, neurogenic bladder, posterior urethral valves (PUV), severe vesicoureteral reflux (VUR) with recurrent urinary tract infections (UTI), and prune belly syndromes. Both the anatomy and the function of the lower urinary tract should be evaluated using ultrasonography (size of the native kidneys and presence/severity of hydronephrosis), voiding cystourethrogram (status of male urethra, VUR, post-void residual volume), urodynamic tests (bladder function), and urethrocystoscopy (in urethral stenosis). Patients must be carefully evaluated to determine those who will require intermittent self-catheterization, pretransplant bladder augmentation, transplant ureterostomy, pretransplant creation of cutaneous urinary conduit using the appendix (Mitrofanoff), or creation of an intestinal (Bricker) conduit (pre- or intra-transplantation surgery). Selection of surgical variants must be individualized and determined by local center experience [9–12]. Native (bilateral or unilateral) nephrectomy performed before or during transplantation is required in selected cases, including children with huge kidneys such as in ARPKD, those with significant polyuria, patients with confirmed WT1 mutations and risk of malignancy, patients with intractable high-grade native proteinuria despite CKD stage 5, children with recurrent UTIs, and children with malignant renovascular hypertension. Pretransplant anuria does not affect outcome. Evaluation of the native kidneys requires ultrasonography with Doppler technique and, in selected cases, CT angiography [13–16].

Vascular Evaluation

Pretransplant evaluation of major vessels (arterial and venous) aims to predict the risk of surgical complications and to plan the placement and vascular anastomoses of the renal graft. The history of pretransplant hemodialysis/hemodiafiltration with use of femoral vascular access, history of thrombotic events, and previous renal transplant(s) are important indications for vascular imaging, including ultrasonography (USG) with Doppler technique, CT angiography, and MR angiography [17, 18].

CKD-Related Comorbidities and Risk Factors

CKD patients develop several uremia-related comorbidities, which may impact the safety and clinical course of renal transplantation, and therefore should be evaluated carefully before listing for transplantation. A high incidence of pretransplant hypertension (HT) and left ventricle hypertrophy (LVH) in up to 70% of children on dialysis is reported. All patients should be evaluated for cardiovascular risk, with use of echocardiography (ECHO) and 24-hour ambulatory blood pressure monitoring. A chest X-ray performed directly prior to surgery serves as a baseline for further monitoring in the pediatric intensive care unit after surgery and general anesthesia. Certain conditions where there is a large abdominal volume that may compromise lung function may warrant a respiratory evaluation prior to surgery. Obese patients should be evaluated in regard to metabolic syndrome (anthropometric evaluation, cholesterol, glucose, and homeostatic model assessment of beta cell function and insulin resistance). The risk of posttransplant diabetes triggered by the use of tacrolimus and steroids can, at least partially, be predicted by metabolic assessment such as a glucose tolerance test before transplantation [19, 20]. Liver function tests should be evaluated routinely, and patients with a history of hepatitis and specific genetic ciliopathies with hepatic involvement require additional workup. This may include USG-Doppler with evaluation of cysts (if relevant) and hepatic blood flow, gastroscopy for esophageal varices, and fibroscan for liver fibrosis. Patients with a history of thrombotic events should be screened for coagulopathies and genetic causes of thrombophilia [21].

Viral status including hepatotropic viruses EBV and CMV should be screened. EBV-seronegativity is common in younger children, and a risk factor for posttransplant lymphoproliferative disease and may influence the selection of immunosuppression [22]. Ideally, children should be fully vaccinated before transplantation. Live vaccines (such as BCG, measles, and varicella) cannot be given in a child on immunosuppression, and measles and varicella can cause very severe illnesses in the immunosuppressed child. Two situations require special attention: one is unsuccessful vaccination against hepatitis B virus, with nonprotective level of antibodies, as immunity is important in this cohort who will have multiple blood tests, surgery, and possible exposure to blood products; and the second is anti-meningococcal immunization, using the most broad spectrum vaccine available in patients who are likely to receive eculizumab prophylaxis or treatment [23, 24]. In children with previous malignancies, the timing and nature of oncologic workup is directed by their underlying condition; however, in general, transplantation is rarely performed until 18 months to 2 years have elapsed after complete remission of the malignancy [25]. Other non-CKD-related comorbidities involving vital organs should be individually analyzed. Irreversible non-renal organ failure may be an indication of multiorgan transplantation (liver-kidney, heart-kidney) and need distinct multidisciplinary assessment. In children with a progressive or life-limiting diseases, especially involving neurologic, metabolic, or mitochondrial cytopathic disorders with poor life expectancy or a very poor quality of life, patients should not be transplanted. This situation needs multidisciplinary assessment and decision-making between the parents and the wider medical team.

Retransplantation

Evaluation of a patient awaiting retransplantation is related mainly to primary graft loss. Recurrence of primary disease such as focal segmental glomerulosclerosis (FSGS) as the reason for graft dysfunction predicts the risk of recurrence in subsequent grafts and may impact the decision for selecting living-related or deceased donor transplantation. In patients with antibody-mediated rejection and graft failure, the severity of HLA sensitization must be evaluated with the mean fluorescence intensity of donor-specific antibodies (DSA).

Patients who require retransplantation should also be evaluated in terms of an indication for graft nephrectomy (versus leaving a graft in situ), preemptive subsequent transplantation, or return to dialysis before retransplantation. In sensitized patients without heavy proteinuria, graft nephrectomy may be contraindicated, as removal of the previous transplant may increase the level of circulating DSA [26, 27]; however, in patients with intractable proteinuria or in cases of graft loss due to BKV nephropathy (when the non-functioning graft may be the source of Polyoma BK virus), the benefit of graft nephrectomy may need to be carefully weighed up against the need for continued immunosuppression. Leaving the previous graft in situ may also pose a challenge for subsequent surgery [28, 29] (Table 1).

Living Donors

Living kidney donation provides one of the two possible organ sources in renal transplantation. It consists of donation of one kidney from a healthy donor benefiting a patient in ESKD. When donation occurs in a child from a parent or close relative, “free and informed” consent from the donor without any monetary incentive or coercion must be confirmed. The donor must be fully informed of and comprehend the risks associated with kidney donation surgery and of those that may occur during the long-term follow-up [30]. This is not a hurdle to the donation itself, but must be considered when psychological evaluation is performed, because the refusal of such a motivated donor may also be associated with negative effects. However, although the emotional link may lead the potential donor to accept greater risks, but donation should not be allowed if there are immediate or long-term medical risks to the donor [30]. This is a responsibility of the transplant center that must make the final decision.

Living kidney transplant offers several advantages for the recipient and may be performed during the final stages of chronic renal insufficiency, even before the need for dialysis is established. This has been demonstrated to be an advantage for the child: dialysis in children may impair cognitive function, growth, bone mineral regulation, cardiovascular disease, and overall life span, compared with transplantation [31]. Moreover, long-term dialysis may also negatively impact on graft and patient survival [31]. An early transplant, as guaranteed by the availability of an organ from a living donor, is therefore highly

Table 1 Evaluation of pediatric candidates for renal transplantation

Indications	Tests and evaluations	Who should be evaluated
Specificity of primary renal disease – cause of CKD	Relevant to disease Genetic, wherever relevant	All cases
Urologic	USG Voiding cystourethrogram Urethrocystoscopy Urodynamic tests	All cases require USG Urethra obstruction History of bladder dysfunction Recurrent UTIs
Vascular	Ultrasound-Doppler CT angiography (of major and renal vessels)	All cases In small(er) children or those with known abdominal pathology
Cardiovascular risk	Echocardiogram Ambulatory BP monitoring Chest X-ray Workup for metabolic syndrome	All cases
Liver tests	Tests for liver function Viral status (hepatitis B, C, and HIV) Gastroscopy Fibroscan	All cases All cases Ciliopathies and chronic liver diseases
Coagulation system	Coagulation panel Genetic screening	Patients with history or risk of thrombotic events or thrombophilia
Oncologic workup	Specific for type of malignancy	Patients with known malignancy
Retransplantation	Sensitization tests: Mean fluorescence intensity (MFI) to donor-specific antibodies (DSA) BKV status	All cases Patients with BK virus nephropathy

desirable for a child in ESKD. The risks to the donor, who is a healthy subject and does not need to have a kidney removed, must be carefully considered, keeping in mind the guiding principle "first do no harm." By donating the kidney, a donor has a small risk of perioperatively death (0.03%, in a large retrospective registry study in the USA of more than 80,000 live kidney donors between April 1, 1994 and March 31, 2009.) [32]. Also, after a longer follow-up (median 15 years), the risk of death for former donors is higher: the hazard ratio for all-cause death is 1.30 [33]. It is mandatory that transplant centers offer an optimal surgical technique, associated with the best outcome in terms of complications and optimal recovery [34]. This requires that the surgeon is well trained in "mini-open" laparoscopy or hand-assisted laparoscopy, which are currently considered the techniques associated with the best outcome [34]. Risk of developing ESKD is not high, yet it is increased when donors are matched with "healthy" non-donors [33, 35], even though absolute numbers remain small. Renal function before donation should be checked carefully, by means of one or more reliable methods: measured creatinine clearance or measured GFR using an exogenous filtration marker (such as inulin, iothalamate, 51Cr-EDTA, iohexol, and 99mTc-DTPA [34, 36]), and patients with a GFR below 60 ml/min/1.73 m^2 should be excluded from donation [34, 36]. In this context, age becomes an important parameter: a mild reduction of renal function in a 60-year-old man is not as significant as in a younger potential donor [37]. Age, thus, becomes a variable to be always included in the evaluation process. This applies to several other issues that may be a cause for late complications, such as renal stones or hypertension. For the latter, for instance, the 2017 KDIGO guidelines suggest accepting a donor who shows blood pressure below 140/90 on treatment with one or two antihypertensive agents [38]. However, even acknowledging the absence of large studies, some papers have showed that blood pressure after kidney donation increases more than normally expected with aging [39, 40]. The development of hypertension is therefore possible over time in a young donor. Even well-controlled hypertension in a young donor probably requires additional caution. This may also apply to other parameters (e.g., proteinuria): detection in a young candidate is more alarming than in an older donor. Classically, older donors rather than young age have been a concern for organ allocation in children. However, most guidelines [34, 36, 41] do not consider older age itself as a cause for excluding a potential organ donor. We should not consider age to be an exclusion criterion per se. The donor should always be fully evaluated, and age is only one of the parameters to be included in the general assessment. A comprehensive list of tests needed to evaluate the suitability of a donor is available and is regularly updated in all existing guidelines [34].

The most recent KDIGO guidelines have proposed a new approach to donor evaluation, based on an "integrative risk-based approach" [34]. This means that, even in the absence of a single clear contraindication, the association of several "negative" parameters might prompt a transplant center to consider excluding a donor. This would be a step forward to improving the long-term outcome of potential kidney donors, but the importance given to each assessment criterion needs to be carefully considered [37, 38]. A young candidate may appear to be a perfect donor just because he has not yet had the time to develop clinical problems, but these may occur over time. This is even more important in a pediatric setting, where young parents of young children, therefore, with a long life expectancy, are being considered as potential donors. The use of risk equations to predict the risk of ESRD in a candidate for kidney donation is recommended [34], but their long-term reliability is still to be demonstrated [42]. It is also important to consider that these predictive methods may be reliable only for the population in which they have been estimated, as significant differences may apply from one population to another [42].

Genetics

Diseases transmitted by means of genetic mutations: Alport syndrome, autosomal dominant polycystic kidney disease (ADPKD), PAX2-mediated CAKUT, and, less frequently, others such as Dent, Frasier, and Gordon syndromes are often encountered in the clinic. In all these cases, the mutation is also present in the parents of the child [40]. In dominant disorders, such as ADPKD or in the X-linked diseases, as in Alport syndrome, the causative mutation must be excluded in the potential donor, whereas, in recessive disorders, usually the candidate donor can be accepted [41, 43]. However, it is always necessary to consider genetic screening and counseling before a final decision is taken. Two diseases, however, deserve some further consideration. In aHUS, a well-known cause for renal failure in children, all efforts should be made to ascertain the mutation in the recipient and in the candidate donor [24]. Transplantation can only be performed if the mutation found in the recipient has an indisputable role in the pathogenesis of aHUS and is not found in the donor [24]. FSGS may occur as a genetic or sporadic form [44]. In the genetic form, parents are obligate carriers and are usually considered as suitable donors, even if, as pointed out by several authors, there is an increased risk for the parents of children with podocin mutations, which is present in combination with the common R229Q variant, which has an allele frequency of 3.7% in the general population. This polymorphism may, in fact, induce a minimal increase of developing a late-onset nephrotic syndrome [45]. This variant should, therefore, be screened before allowing a kidney donation, and, in the case of positivity, the opportunity and the risk of allowing the donation should be carefully discussed with the candidate. In the sporadic forms, no risk for the donor is present; however, a possible relapse may be expected in almost one-third of the patients [44], and, if a first transplant is lost due to relapse, the risk for a new failure becomes much higher [46]. Most of the existing guidelines do not exclude LRD in children with FSGS in the first transplant, whereas a second transplant should be allowed only in specific conditions [34, 38, 47]. More recently, the importance of the risk associated with the presence of variants of the APOL1 alleles has been highlighted. However, the meaning of analyzing these risk variants is still unclear. They are common in persons of sub-Saharan African descent. Approximately 13%

of African Americans carry two APOL1 risk alleles. This condition increases the risk of FSGS, proteinuria, reduced GFR, younger age on dialysis, and more rapid progression of kidney disease [34, 38]. The presence of two APOL1 risk alleles in a deceased donor associates with two to four times higher RR of graft loss in the recipient, compared with 0 or 1 risk alleles [34, 48]. There is currently not enough information to suggest a definite plan for this issue, and a large study has just been launched to ascertain the actual importance of this phenomenon [49].

ABO Incompatible

A possible hurdle in the search for a compatible donor is the ABO-incompatibility between the candidate for transplantation and the potential donor. In these cases, two options exist: ABO-incompatible kidney transplant or paired kidney donation (crossover transplantation). The first option is widely used in the adult setting with excellent results, although still inferior to ABO-compatible control patients [50]. Even if results are considered satisfactory, there is still excess mortality and loss of kidney grafts compared with ABO-compatible transplants within the first 3 years after transplantation [51], and a risk from increased immunosuppression. In children, good results are reported [52] but the procedure is more invasive than that needed for compatible transplantations, as the ABO-incompatible desensitization protocols require preemptive removal of isoagglutinin by means of plasmapheresis or immunoadsorption, and specific conditioning protocols, which most often include anti-CD20 agents [51]. The alternative is paired kidney exchange, where two noncompatible donors swap their kidneys in order to let both their candidates receive the organ. This may be done as a direct donation between two couples or after longer chains of donors/candidates. The efficacy of this practice mainly depends on the dimension of the donor's pool, thus nationwide programs are desirable [53], but programs involving more than one country have also been proposed [54]. Moreover, it is important that this system considers a possible rescue system for the transplantations that show a primary nonfunction, in order to increase the willingness of patients to participate. Several systems guarantee the prioritization of an organ allocation in a future end chain or from the deceased donor waiting list in case of early failure [53, 55].

Posttransplant Monitoring of the Donor

It is important that post-donation monitoring is the responsibility of the transplant team [34, 38], which should guarantee at least a yearly monitoring of renal function and blood pressure. Also, it is important to stress the need for each former donor to implement healthy lifestyle behaviors [34]. A pediatric center might also decide to associate with an adult nephrology center for donor monitoring for logistic reasons.

The Deceased Donor

Pediatric Placement on a Deceased Donor List

Most children, as compared to adults with ESKD, are listed for transplantation. The placement of a child on a kidney donor list varies across countries. In most countries the allocation of deceased kidneys is governed by a state or national allocation group. In some cases, this is a nongovernment body, such as the USA, where this is contracted out to United Network for Organ Sharing (UNOS). Donors may be either brain dead or have suffered cardiac death. The definition of these is closely regulated in most jurisdictions. Consent to donate varies in different countries, with some having an “opt-in” system, requiring evidence of prior intent to donate or consent from relatives; however, in other countries, an “opt-out” system exists. The relative benefits and ethical principles underlying each model remain a subject of continued discussion.

Donor lists are created using blood group and mostly comprise waiting time on dialysis and closeness of HLA matching between the recipient and the kidney donor. For highly matched kidneys where the two major Class I alleles A and B and the major Class II allele have a 6 out of 6 match, many jurisdictions will give priority for allocation of that "full" match in their jurisdiction and may also give it priority nationally or across national boundaries in places such as Europe.

Blood Group Issues

Historically, kidneys were thought to only be able to be given to recipients who did not carry antibodies to the blood group of the donor kidney. This meant that blood group O kidneys could be donated to all other blood groups and their own, whereas AB kidneys could only be given to patients who were blood group AB. Given this situation, most allocation for deceased kidneys is done within blood groups.

ABO mismatched transplantation has been discussed in the living donor section, and, although theoretically possible, ABO incompatible deceased donors are rarely used for pediatric recipients [52].

Immune Matching

As well as waiting time, most centers give importance to the degree of immune matching between donor and recipient on the deceased donor waiting list. Long-term data shows that HLA identical kidneys do best, and that survival of donor kidneys becomes progressively worse with worse matching [54]. Further Class II matching is probably more important than Class I matching. Increasingly, specific antigenic peptides on the surface of HLA molecules, called eplets, are being evaluated as they potentially give a better risk score than the HLA mismatches alone [56, 57]. Historically, HLA testing was done using recipient sera to screen for anti-donor antibodies and measurement of the HLA on the donor kidneys. Donor and recipient HLA typing is now done by rapid molecular methods by virtual cross-match using flow cytometry with HLA-antigen-specific beads. This allows identification prior to acceptance of a donor or the presence of donor-specific antibodies, where the amount of antibody is measured by the mean fluorescence intensity of antibodies binding to the beads. For two reasons, the degree of matching is less of a component in allocation than it was initially thought. The first is the issue of equity, where patients from rare ethnic groups would wait longer because of greater mismatches with the pool of donors. The second is the improvement in immunosuppression, reducing the risk of rejection, even in mismatched grafts [58].

Quality of the Kidney from the Deceased Donor Pool

As well as immune matching, a number of factors relating to the quality of the kidney from the donor waiting list must be considered. The most commonly assessed factors can be considered from the kidney donor profile index that combines a number of donor factors into a single number that summarizes the likelihood of graft failure from a deceased donor. This is dominated by the age of the donor, but includes a number of infectious and vascular risks. The greater the age difference between donor and recipient, the worse the outcome [59]. Genetic factors may also predict a transplant kidney's risk of developing graft fibrosis [60]. The degree of acute kidney injury and delayed graft function can have a significant effect on patient and graft survival [61].

Infectious Risk

Potential pathogens, including Hepatitis B, Hepatitis C, and HIV, are a serious risk to the recipient and must be screened for using both serology and rapid molecular diagnostics. In addition, the donor's CMV and EBV serological status should be determined. The presence of a CMV-positive kidney will require prophylaxis and/or treatment with antiviral agents such as valganciclovir or valcyclovir for 6–12 months [62]. EBV-positive kidneys carry the risk of both EBV-related disease and the future development of PTLD and even lymphoma [63]. The presence of either of these viruses or of the BK virus requires close monitoring and appropriate treatment for the recipient [64].

Marginal Donors

To enlarge the donor pool, "marginal donors" are being used. These includes non-heart-beating donors or donation after cardiac death, older donors, and very young donors, including "en bloc" donation [65]. Other areas of higher risk include donations from patients with certain malignancies, or donor kidneys with a resectable tumor [66]. Infectious risk is an area which has become greater with the increasing number of opioid-dependent donors [67]. These donors have a risk of blood-borne viruses, including HIV and Hepatitis C. In many cases, the risk is quantifiable and low, but recipients (and/or their legal representatives) must be fully informed of the associated risks and appropriate consent taken.

Currently, it is not possible to perform any manipulations on a donor kidney prior to the cessation of the donor's life. A major intervention that has been applied is the use of cold, and, more recently, warm pulsatile perfusion to support a kidney prior to transplantation and possibly to improve its function. Pharmacological manipulation of the kidney to improve graft life and function may also be possible [68]. Of particular interest is the smaller donor. Smaller children on dialysis may do better in the long term with a smaller kidney that allows earlier transplantation. There is early tantalizing data that smaller kidneys may have the capacity to grow with their recipient [69]. However, the risk of graft thrombosis in the small kidneys must be balanced against the benefits of earlier transplantation, and these transplants can only be performed in centers with the requisite surgical and medical expertise.

Equity Versus Utility

As a precious resource, there is significant pressure to maximize the years off dialysis provided by a donor kidney. This is balanced by the need to have equitable access for recipients, regardless of race, sex, or socioeconomic status. Pediatric recipients may have additional comorbidities such as blindness, as well as cognitive or physical disabilities, where access to a kidney transplant requires advocacy from their team [70].

Looking After Children

In many countries preference is given to children over adults on the kidney transplant waiting list, prioritizing them either at the commencement of dialysis or after a period of time on dialysis [71]. In some, this is by giving children the equivalent time on dialysis to move them up the list; in other areas, it is by offering them younger or better matched kidneys – in some cases, trying to match for HLA Class II because of better potential long-term outcomes associated with this [72]. The definition of "pediatric" recipients varies between jurisdictions, with some giving preference to 15 and below and some to 18 and below. Increasingly, there is a desire to also consider some advantage for young adults who may have a similar justification for access to a transplant.

Determining the Suitability of a Deceased Donor Kidney for a Particular Recipient

For most children, the decision around a potential donor will involve a nephrologist and a transplant surgeon weighing up the quality of a particular donor against the potential risks. In general, most physicians will receive a large amount of data on the donor, including infectious risks, HLA matching, the presence of DSAs, and associated morbidities of the donor, such as weight, hypertension, diabetes risk, smoking, kidney function, appearance of the kidneys, and vascular information such as the number of vessels and the presence of CMV, EBV, and other infectious agents [73]. Once a decision to go ahead is made, the coordination of a retrieval team including clinicians, surgeons, intensivists, and nursing and allied health workers is required for the fastest possible transport of the kidney to the recipient. There are many models that are successful, but high volume, ongoing review, and expertise of the retrieval team are often the basis for the best outcomes [74].

Multiorgan Transplants

Multiorgan transplants require a multidisciplinary team approach to coordinate the decision about appropriateness and timing of surgery. The listing for combined organs for children generally involves the need for a larger pool of potential donors and agreement for the use of combined organs to be prioritized over individual use. In both ciliopathies and certain metabolic diseases, multiorgan transplants have improved patient survival and quality of life [75–77].

Summary

Pediatric recipients of kidney transplants are a special group, with unique recipient- and donor-related issues. Improving transplant outcomes depends on a careful evaluation of the pediatric recipient and their potential donor in order to improve the quality of life and life expectancy to these children.

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Renal Transplantation: Surgical and Perioperative Management

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Britta Höcker and Jodi M. Smith

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Abstract

Kidney transplant is the treatment of choice for children with end-stage kidney disease due to the beneficial effects on growth and development. Fundamental to the success of the transplant procedure is the close collaboration among multiple disciplines including transplant surgery, transplant anesthesia, pediatric intensivists, and transplant nephrology. Awareness of the key surgical and urologic issues as well as the potential complications helps guide early posttransplant management in order to optimize outcomes.

Keywords

Transplant organs · Living donor kidney transplant · Deceased donor kidney transplant · Native nephrectomy · Anticoagulation · Posttransplant management · Delayed graft function

Introduction

While kidney transplant is known to be the least complex transplant procedure, it does not forgive technical errors and brings unique challenges in

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the pediatric recipient. The young pediatric recipient may be especially vulnerable to transplant complications emphasizing the necessity of meticulous surgical technique and attentive post-transplant management [1, 2].

In this chapter, we discuss the surgical and urologic procedures used in pediatric kidney transplantation. In addition, hemodynamics, perioperative fluid and electrolyte management, anticoagulation, and strategies for infection prevention are discussed. Also, postoperative complications such as graft thrombosis, renal artery stenosis, lymphoceles, as well as urologic obstruction or leakage are highlighted.

Surgical Management

Back-Table Preparation of the Donor Kidney

The anatomic structures transplanted during deceased donor kidney grafting are composed of the kidney with perinephric fat, the renal artery with a cuff of the donor aorta (Carrel patch), the renal vein with a cuff of the inferior vena cava, and the ureter with periureteral tissue. The kidney which is first flushed with iced preservation solution is then immersed in an iced solution (such as saline, Ringer's lactate or histidine-tryptophan-ketoglutarate solution) to prevent warm ischemia. Perinephric fat is removed in the course of back-table preparation. In the event of multiple vessels, reconstruction may be required prior to grafting. Whereas a small upper pole vein may be ligated owing to a sufficient collateral venous drainage, the whole arterial supply must be revascularized in the absence of a collateral arterial circulation of the kidney. Careful handling of the ureter and periureteral tissue is of vital importance to avoid inferior blood supply to the ureter and subsequent ureteral necrosis. In the case of living donation, the donor artery and vein are shorter because the vena cava and aorta are obviously unavailable [3].

Operative Technique in Older Children and Adolescents

The transplantation procedure for children weighing more than 20 kg is usually the same as that for adults. The pediatric patient is brought to the operating room to receive general anesthesia and a central venous line is placed. If there is a concern that delayed graft function may occur, a double-lumen central venous line suitable for postoperative hemodialysis should be placed. An additional arterial line can be placed percutaneously if invasive blood pressure measurement is required for hemodynamic monitoring in an unstable circulatory situation. The patient is put into supine position and a urethral catheter is placed. The bladder is irrigated and distended with sterile saline [4, 5].

In contrast to other transplant organs, the kidney is placed heterotopically, usually retroperitoneally in the right iliac fossa because of the more superficial and horizontal course of the right iliac vein. However, some surgeons favor transplanting the kidney contralaterally, which means placing the right kidney in the left iliac fossa and vice versa, whereas others prefer placing the kidney ipsilaterally. Of course, pretransplant surgeries, multiple transplants, and nephrectomy of the native kidney must be taken into consideration when choosing the transplant bed.

Occasionally, native nephrectomy is needed at the time of transplant. Indications for nephrectomy include (i) need for space in small children or in the setting of enlarged kidneys such as polycystic kidney disease; (ii) uncontrolled renal related hypertension; (iii) recurrent infections in native kidneys; (iv) polyuria; and (v) nephrotic syndrome. Native nephrectomy is often needed in patients with active nephrotic syndrome, either congenital or acquired. It is suggested that native nephrectomies are performed prior to transplant in order to allow time for the hypercoagulable physiology to correct to minimize the risk of vascular thrombosis [6]. In some centers, a unilateral nephrectomy is performed months to years before transplantation, with the second kidney removed at the time of the transplant surgery while in others, both are removed prior to transplant.

The typical incision is a modified lower abdominal hockey-stick type, with minimal muscle-splitting, or like the so-called Gibson incision, which extends from the midline above the symphysis pubis in curvilinear fashion to a point two finger-breadths medial to the anterior superior iliac. The inferior epigastric vessels and lymphatics are ligated and divided, and exposure of the iliac fossa is obtained by reflecting the peritoneum medially. In males, the spermatic cord is preserved, and in females, the round ligament is divided. By placement of a stationary retractor, the external iliac and common iliac vessels are exposed and dissected. It is left to the surgeon's discretion to perform heparinization in close cooperation with the anesthesiologist. The renal vein is usually anastomosed first, to avoid insufficient perfusion of the lower extremity. After placement of vascular occlusion clamps, the venous anastomosis is performed between renal donor vein and recipient external or common iliac vein in an end-to-side fashion using a running 5–0 or 6–0 polypropylene suture. The arterial anastomosis is usually done between the donor renal artery and the recipient external or common iliac artery in an end-to-side mode using a running 5–0 or 6–0 polypropylene suture. The arterial orifice can be facilitated by means of a cardiac punch. In case of multiple renal arteries, an aortic Carrel patch or side-to-side anastomosis of equalized renal arteries may be needed. To promote diuresis, mannitol and furosemide are usually administered intravenously. Before reperfusion of the transplant kidney, it is essential to establish sufficiently high systolic blood and central venous pressures. Upon completion of the anastomoses, the vascular clamps are removed. Hemostasis should be ascertained by examining the anastomoses, capsule, and hilar fat. Attention then has to be focused on the ureter [3, 5]. Urinary continuity may be restored by different techniques which will be discussed below (see subchapter "Urologic Management").

Surgical Considerations in Young Children and Small Infants

In children weighing 10–20 kg, an extraperitoneal approach is still feasible, but anastomoses are usually done in an end-to-side fashion between renal vein and inferior vena cava or common iliac vein as well as between renal artery and aorta or common iliac artery. In small infants under 10 kg, most surgeons use an intra-abdominal approach. A midline longitudinal abdominal incision is performed extending from the xiphoid to the pubis. Cecum and ascending colon are reflected medially to expose the aorta and vena cava inferior. Aggressive volume replacement is necessary at the time of reperfusion since a large adult donor kidney claims a significant portion of the normal pediatric blood volume [4, 6–8].

Few transplant surgeons choose an extraperitoneal approach, even for small infants, to exclude bowel complications and to enable peritoneal dialysis after renal transplantation, if required, and an easier access to transplant biopsy. Vitola et al. reported on a study in 62 patients weighing less than 15 kg, in whom an extraperitoneal approach proved most successful [9]. Recently, Alameddine et al. presented a method where anterior mobilization of the recipient liver and kidney is accomplished through blunt dissection of the renal fossa. It allows sufficient space to be created for the adult renal allograft to lie retro-hepatically and to occupy the space from the diaphragm to the bladder. This method yielded excellent results in a single-center study in 21 pediatric patients [10]. A recent analysis of the *Cooperative European Paediatric Renal Transplant Initiative* (CERTAIN) registry evaluated the outcome of kidney transplantation in small infants. In that cohort, renal grafts from adult donors were transplanted only retroperitoneally with no increased risk of graft compression [11].

There are several reports of renal transplantation in infants weighing less than 10 kg [12–15]. Royo Gomes et al. studied the outcome of 10 kidney grafts in 9 infants weighing 7 kg on average [13]. All were placed retroperitoneally. Three of

10 grafts were lost; 2 due to acute rejection and recurrence of the primary renal disease, respectively; and 1 due to ischemia within the first 48 h. The authors concluded that kidney transplantation is feasible in low-weight patients despite technical difficulties. In addition, Singer et al. compared the outcomes of renal transplantation in 245 children weighing 8 to <10 kg with that of 804 patients weighing 10–13 kg [14]. They found no significant difference in graft survival between both groups [14]. Another single-center study showed similar patient and graft survival in renal transplant patients weighing <11 kg and those weighing >11 kg. Nevertheless, the authors emphasized the importance of aggressive fluid resuscitation and attention to perioperative hemodynamics to avoid graft thrombosis in very small children [15].

Urologic Management

Continuity of the urinary tract is usually achieved by neoureterocystostomy. The transplant ureter should be trimmed of devascularized tissue and tunneled under the cord structures without twisting. Use is made of different methods of neoureterocystostomy [3, 5, 6].

A common approach, termed Lich-Gregoir technique, consists in reimplanting the ureter extravesically into the bladder. This approach was originally intended for correction of vesicoureteral reflux and was introduced in kidney transplantation in 1968. The muscular layer of the bladder is divided to expose the bulging mucosa. Upon opening of the mucosa, an anastomosis is performed between the ureteral and bladder mucosa, a muscular tunnel of 2–3 cm length being closed over the mucosal anastomosis to prevent vesicoureteral reflux. Care has to be taken to avoid ureteral strangulation. The advantages offered by this extravesical approach are a smaller bladder cystostomy and a resultant lower risk of contamination and urinary leakage.

The transvesical Leadbetter-Politano method is based on anterior cystostomy. A submucosal tunnel is created, the ureter brought into the bladder posteriorly via a separate opening and sewn into the bladder from the inside. Afterwards, the bladder is closed by a watertight cystorrhaphy using a three-layer closure. The benefit of this method is a low risk of reflux, which may be accompanied, however, by ureteral obstruction.

Another viable technique of neoureterocystostomy is to create a nipple valve, where a nipple can be formed by turning the end of the ureter out on itself, placing the end of the nipple into the bladder, and fashioning the turned-back mucosal edge of the ureter to the recipient bladder mucosa with a running mucosal suture line. This technique involves a low rate of reflux but a higher incidence of obstruction than the aforementioned approaches [3, 7, 16].

About one-third of pediatric kidney transplant recipients have congenital anomalies of the kidney and the urinary tract (CAKUT) as the primary cause of renal failure. In addition, conditions such as neurogenic detrusor dysfunction secondary to meningomyelocele, bladder exstrophy or primary anuria with a subsequent minimal bladder volume may require reconstruction of the urinary tract even prior to transplantation. Depending on the individual anatomical situation prevailing, the donor ureter may be anastomosed with an augmented bladder or an ileal conduit.

Since opinions on the suitability of ureteral stents do diverge, their use is left to the surgeon's discretion. Some surgeons prefer a so-called single-J stent, which is placed in the renal pelvis and usually removed after 5–7 days posttransplant. In complex anatomical situations where the risk of ureteral obstruction is high, a double-J stent may be inserted, which extends from the renal pelvis to the bladder, and should usually be removed after 6–8 weeks posttransplant. Using a suprapubic urine catheter and a single-J stent may facilitate the distinction between urine output of the native kidney and that of the renal allograft [3, 7, 16].

Posttransplant Management

Early Postoperative Management (Table 1)

Initial assessment of the transplant recipient upon arrival from operating room includes assessment of hemodynamic and respiratory stability. Most

Table 1 Immediate postoperative assessment

Recipient information
Etiology of end-stage renal disease and any special considerations (ex. re: Risk of recurrent disease how does that change monitoring posttransplant ex. check daily protein/creatinine ratio for recurrent FSGS) Special urologic considerations such as vesicostomy, bladder augment, or mitrofanoff Pretransplant blood pressures and antihypertensive medication regimen Urine output pretransplant Preemptive transplant or dialysis pretransplant
Transplant information
Living donor or deceased donor Cold ischemia and warm ischemia time
Hemodynamic assessment
Blood pressure target parameters Low BP goal that would require fluid bolus High BP goal that would require prn antihypertensive Set target CVP
Discuss urine output parameters to call for such as low urine output, gross hematuria
Fluid management plan
Anticoagulation plan and target
Immunosuppression plan; timing of induction and maintenance immunosuppression medication
Infectious prophylaxis
Pain management
Lab monitoring plan

children are extubated immediately posttransplant with the occasional exception of the youngest recipients. Sign out from the anesthesia and OR team typically includes details on the intraoperative blood pressures and CVP, volume administered, metabolic correction, urine output, and any bleeding or need for intraoperative transfusion. The surgeon reports the cold and warm ischemia times and any surgical concerns. This is a good time for the care team to establish goals for the next 24 h such as target low and high blood pressure goals for which fluid boluses or prn antihypertensives should be administered. Target CVP goals are established at this time in addition to goals for urine output. Awareness of the patient's pretransplant blood pressure and antihypertensive regimens as well as native kidney urine output is important to help guide posttransplant management. Anticoagulation management plans are discussed with target levels depending on the patient's thrombosis risk. Review of the immunosuppression plan with details on the timing of induction and maintenance medication initiation are discussed. Renal Doppler ultrasound is performed in the immediate postoperative period to confirm graft vascular flow.

Hemodynamics

Attention should be focused on avoiding severe hypertension and hypotension in the immediate postoperative period. Invasive blood pressure monitoring via arterial line may be necessary in the first 48–72 hours. Blood pressure parameters, both high and low, should be established. In general, relatively higher blood pressures in the immediate postoperative period are tolerated to avoid renal ischemia. Knowledge of the patient's pretransplant blood pressure ranges is important to guide individualization of posttransplant goals.

Postoperative hypertension due to high volume administration and medications (steroids and calcineurin inhibitors) can be problematic, adding stress on the renal arterial anastomosis and increasing the risk of hypertensive seizure. However, caution should be used in the treatment of hypertension in the initial postoperative period. Blood pressures between 90th and 95th percentile for age, gender, and height may be tolerated depending on the clinical situation. The agents of choice for treatment of hypertension in the immediate postoperative period include short-acting vasodilators, beta blockers, or calcium channel blockers. In general, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists are avoided in the early posttransplant period as they may induce renal insufficiency in the setting of diminished effective arterial blood volume. Diuretics may be helpful if hypervolemia is contributing to hypertension. Awareness of the patient's pretransplant antihypertensive regimen is helpful to guide posttransplant medication choices particularly if the regimen included medications such as beta blockers or clonidine that can be associated with a risk of rebound hypertension when stopped. If continuous infusion of antihypertensive medication is required, caution should be used with nicardipine as it may inhibit tacrolimus metabolism by cytochrome P450 resulting in overexposure and potential toxicity [17].

Hypotension and under perfusion of the adult-sized kidney in the pediatric patient is equally problematic. The treatment of choice for hypotension posttransplant is volume expansion with crystalloid and/or colloid. If possible, vasopressors are avoided, given the risk of renal vasoconstriction. An adult kidney in a small child needs a normal to high blood pressure and good intravascular volume for adequate perfusion. If the child has one or both native kidneys, one should be alert to hypotension and dehydration from polyuria.

Fluid and Electrolyte Management

Meticulous fluid and electrolyte management is critical for pediatric kidney transplant recipients. Additional intravascular volume repletion in the perioperative period is important to establish diuresis and avoid delayed graft function. Small children may require massive volume expansion to maintain adequate perfusion of the adult-sized kidney. It may be prudent to keep the small child intubated in the early postoperative phase if there are any signs of pulmonary edema. Central venous pressure monitoring is used to help assess intravascular volume status.

Intravenous fluid management posttransplant may include insensible fluids (300–400 ml/m^2/day) in addition to urine output replacement. Urine output is replaced with intravenous (IV) fluid, usually ½ normal saline with dextrose and water. In some cases, the postoperative diuresis is so large that hyperglycemia can result when 5% dextrose is used for urine replacement. Urine output is usually replaced mL/mL throughout the first 24 h with a gradual tapering of IV urine replacement as the patient's oral intake increases.

Supplemental loop diuretics or osmotic agents (mannitol) may be needed to facilitate diuresis in the oliguric patient. Although "renal dose dopamine" (3–5 mcg/kg/min) or other inotropic support to enhance renal vasculature dilatation was practiced by some centers, it is no longer considered beneficial. Providers should carefully administer medications associated with cytokine release (such as polyclonal and monoclonal T cell antibodies) in the patient who is volume expanded. Excessive volume administration may exacerbate hypertension. High volume diuresis may deplete electrolytes depending on the type of fluid replacement administered. At time of transplant, most patients have normal or elevated serum phosphorous levels. In the setting of delayed graft function, phosphate binders may be necessary to control hyperphosphatemia. With improved urine output, phosphaturia occurs due to hyperparathyroidism and/or the effect of calcineurin inhibitors. The goal phosphate level depends on the age of patient with higher acceptable levels in the young growing child. Oral phosphate supplements include preparations that contain potassium phosphate or a combination of sodium and potassium phosphate in either tablet or powder form. The choice of supplement depends on the patient's serum potassium and palatability. Hypomagnesemia may occur due to calcineurin inhibitor-induced renal magnesium wasting. A complication often seen with oral magnesium containing preparations is diarrhea. When this occurs, a dose reduction is necessary. In the patient who has delayed graft function, avoid magnesium-containing antacids to prevent the development of hypermagnesemia. Supplemental sodium bicarbonate may be needed in the postoperative period, especially in those with delayed graft function. Patients who are undergoing substantial diuresis and receiving high volume IV fluid may also need addition of bicarbonate containing solution to avoid metabolic acidosis.

Glucose management can be a problematic, especially in the pediatric patient with type I diabetes mellitus or in the patient on tacrolimus. An insulin drip may be required to maintain glycemic control. Once on a stable diet, patients can be switched to subcutaneous administration of long-acting insulin with a sliding scale of a short-acting preparation. Steroids complicate glycemic control and increased doses of insulin may be needed, especially when the patient is on higher doses of steroids in the first few months after transplant.

Urine Output

Close monitoring of urine output is very important in the immediate posttransplant period. In general, a Foley catheter is usually kept in the bladder for the first few postoperative days to avoid stressing the ureterocystostomy as well as to facilitate hourly assessment of urine output. It is important to ensure the Foley catheter in place and patent. Some centers also use suprapubic catheters. If the catheter is inadvertently discontinued or is not draining well, immediate evaluation is indicated. In most cases, the Foley will need to be replaced emergently to keep the bladder decompressed and to prevent tension on bladder-ureteral anastomosis.

Urine output in the early posttransplant period can range from oligo-anuria to polyuria and fluctuates depending on the clinical situation. Awareness of the patient's pretransplant urine output is useful in order to assess the adequacy of the urine output early posttransplant. The first step in addressing low urine output is to evaluate the patient's intravascular volume status (central venous pressure [CVP], blood pressure), in addition to ensuring patency of the Foley catheter. If the patient is hypovolemic, volume expansion with 10–20 mL/kg of isotonic saline can be administered. It is common for children to receive multiple fluid boluses in the first 24 h posttransplant. In contrast, if the patient is evaluated to be hypervolemic, a dose of intravenous furosemide can be given. If urine output does not improve with fluid resuscitation and diuretic challenge, a renal ultrasound with Doppler should be performed to confirm flow to the graft and assess for obstruction.

Reasons for a decrease in urinary output include volume depletion, urologic complications such as urine leak or obstruction, vascular thrombosis, calcineurin inhibitor nephrotoxicity and rejection. Urine leak or lymphocele is indicated by abdominal pain, distention, elevated creatinine and blood urea nitrogen (BUN), and fever. Ureteral stenosis or obstruction is indicated by decreased urine output/anuria, elevated BUN and creatinine, and pain over graft.

Gross hematuria may occur posttransplant due to the use of anticoagulation. However, there are some serious complications that merit consideration. The differential diagnosis of gross hematuria includes renal vein thrombosis, renal artery thrombosis, infection, ureteral stent related, recurrent disease, and thrombotic microangiopathy. Rapid evaluation with blood work, urine analysis, and microscopy, renal ultrasound with Doppler and possible biopsy is recommended.

Infection Surveillance and Prevention [18–20]

The use of perioperative intravenous antibiotics is transplant center specific. Many centers use a first-generation cephalosporin for the first 24–48 h in the nonallergic patient. Close daily attention to the patient's vital signs and physical examination is critical including evaluation of wounds, indwelling catheters, and drains. Lines, drains, and urinary catheters should be discontinued as soon as medically indicated to avoid risk of infection. Urine should be cultured and the sediment examined routinely as part of posttransplant management. Work-up for suspected infection in the pediatric kidney transplant recipient should include: physical examination, cultures (urine, blood, drains, and peritoneal fluid if indicated), chest X-ray, cytomegalovirus (CMV) and Epstein–Barr virus (EBV) polymerase chain reaction (PCR), and consider spinal tap if indicated.

Prophylaxis against *Pneumocystis jirovecii* using trimethoprim – sulfamethoxazole (TMP/SMZ) is indicated if the patient is not allergic. If the patient is TMP/SMZ allergic, dapsone or pentamidine can be used. Oral antifungal prophylaxis may be given for the first 1–3 months after transplant, usually in the form of nystatin, or clotrimazole lozenges, or fluconazole. Adjustment and close monitoring of the levels of calcineurin inhibitor is necessary when fluconazole is used due to the drug–drug interactions wherein the fluconazole inhibits the calcineurin inhibitor metabolism by cytochrome P450. Consensus recommendations guide the duration of therapy of viral prophylaxis for CMV with ganciclovir or valganciclovir based on the serostatus of the donor and recipient [19–20]. For CMV D+/R- patients, 3–6 months of prophylaxis with oral ganciclovir or valganciclovir is recommended. For CMV R+ patients, 3 months is recommended but 6 months should be considered if anti-lymphocyte induction is used. No prophylaxis is recommended in the CMV D−/R- patient. The dose for ganciclovir/valganciclovir needs to be adjusted according to the estimated creatinine clearance.

Anticoagulation

Screening for inherited and acquired thrombophilic risk factors is not routinely performed in all transplant recipients and is transplant center dependent. Indications for screening include family history of clotting and/or prior episodes of thrombosis. Preoperative screening typically includes assessment for protein C, S or antithrombin III deficiency, factor V Leiden, prothrombin gene mutations, and the presence of antiphospholipid antibodies. The use of prophylactic therapy with heparin, low-molecular-weight heparin, and/or aspirin to prevent thrombosis also varies by transplant center. Indications for the use of prophylaxis include identified thrombophilic risk factors, *en bloc*/young donor age, and difficult vascular reconstruction. In some centers, anticoagulation therapy is used prophylactically in all pediatric kidney transplant recipients. The duration of therapy generally varies from 3 months to 6 months depending on the indication.

A recent systematic review aimed to determine the effectiveness and safety of antithrombotic prophylaxis in pediatric and adult kidney transplant recipients evaluating 12 studies comprising 2370 patients were included in the evaluation [21]. The overall risk for developing renal graft thrombosis was lower in the group with antithrombotic prophylaxis compared to the control group (risk ratio 0.24, 95% confidence interval 0.12–0.49). Heparin was the preferred drug, followed by acetylsalicylic acid or a combination of acetylsalicylic acid, heparin, and dipyridamole. Adverse effects were reported rarely, with minor bleeding as the main complication.

Posttransplant Complications

Acute Kidney Injury

Several factors can contribute to posttransplant acute kidney injury. These include donor type and age, technique of organ preservation, adequacy of volume replacement during and after surgery, and cold and warm ischemia times. Tubular damage may be aggravated by the use of cyclosporine or tacrolimus. Recovery of renal function can be divided into three broad categories: rapid recover of graft function, slow, or delayed. Table 2 summarizes causes of graft dysfunction. Delayed graft function has a specific definition and refers to the need for dialysis in the first week posttransplant. This occurs in less than 10% of pediatric kidney transplant recipients [22]. Indications for dialysis posttransplant include electrolyte abnormalities and/or significant fluid overload leading to pulmonary compromise and hypertension. Caution should be used when initiating dialysis in the early posttransplant period to avoid significant intravascular volume depletion that can compromise renal perfusion.

Surgical Complications

Graft Thrombosis

Graft thrombosis may be either arterial or venous and usually develops within the first 3 days posttransplant, its incidence ranging between 0.5% and 8%. Because thrombosis is facilitated by a previous thrombotic tendency, thrombophilia screening of transplant recipients prior to surgery is as essential as is antithrombotic management, which will be discussed below. Early thrombosis may be due to surgical techniques. Clinical symptoms are, among others, cessation of urine output, swelling of the transplant kidney, tenderness, graft function deterioration, and hematuria. Diagnosis is possible by means of Doppler ultrasound. Unfortunately, confirmed thrombosis usually requires graft

Table 2 Causes of graft dysfunction[a]

Causes of graft dysfunction	How to assess	Suggested management
Volume depletion	Fluid balance/weight CVP and hemodynamic assessment	Fluid bolus
Acute tubular necrosis	Urine analysis	Close monitoring of fluid balance and hemodynamics
Vascular complications: Renal artery stenosis Renal vein thrombosis	Urgent transplant ultrasound with Doppler interrogation	Immediate surgical evaluation/ intervention
Urologic: Urinary obstruction Urinary leak	Imaging: Transplant ultrasound to look for collecting system dilation Nuclear medicine study to assess for urinary leak Body fluid creatinine assessment	Immediate surgical evaluation/ intervention
Calcineurin nephrotoxicity	Drug levels Kidney transplant biopsy	Pharmacokinetic studies Assess if any drug interaction Consider adjustment of maintenance therapy
Hyperacute rejection	Donor-specific antibodies Kidney transplant biopsy	Treatment of rejection

[a] Recurrence of primary disease and thrombotic microangiopathy are discussed in separate chapters

nephrectomy, although rare cases of successful graft salvage by thrombectomy and fibrinolysis are reported in the literature [3, 17, 18].

Renal Artery Stenosis

Renal artery stenosis is the most common vascular complication experienced after kidney transplantation. It occurs late, usually between 3 months and 2 years posttransplant, its incidence ranging from 2% to 10%. Refractory arterial hypertension and graft dysfunction are typical clinical signs of renal artery stenosis, which usually develops in the area of the anastomosis. Its causes may be technical error, kinking of the vessel and sometimes artherosclerotic plaques in older recipients, just like hyperplastic response of endothelial or smooth muscle to occult intraoperative trauma and proliferative responses to immunological stimuli. It can be diagnosed by means of Doppler ultrasound, CT, NMR angiogram, or arteriogram. Treatment consists in percutaneous transluminal angioplasty with balloon dilatation or placing of stents. Sometimes surgical correction is necessary as well [3, 7, 23, 24].

Hemorrhage

Postoperative bleeding is a rare complication following kidney transplantation. Early hemorrhage may arise from latent small vessels in the renal hilum, which did not become apparent at the time of reperfusion, due to vasospasm. Hence, meticulous preparation of the allograft and hemostasis are of major importance. Late hemorrhage may occur as a result of mycotic aneurysm ruptures or perianastomotic infection. Clinical signs of hemorrhage may be intense graft site or back pain, accompanied by a drop in hematocrit, and hemoglobin, and by symptoms of vascular collapse. In such events, immediate surgical intervention is required to prevent life-threatening bleeding [3, 7].

Lymphocele

Lymphoceles are lymph collections between the inferior kidney pole and the bladder, which develop due to insufficient ligation of afferent lymphatics overlying the iliac vessels. Their incidence ranges between 0.6% and 18%. Most lymphoceles are small and asymptomatic.

However, some of them may lead to clinical symptoms such as leg swelling indicative of deep vein thrombosis, abdominal mass, ureteral obstruction, and graft dysfunction due to hydronephrosis. Occasionally, lymphoceles may cause incontinence secondary to bladder compression, scrotal mass, or cutaneous lymph fistulae. Lymphoceles are identifiable by means of ultrasound as roundish, sonolucent, septated mass. Needle aspiration of the fluid does confirm diagnosis if the fluid is clear and has a high protein content, whereas the creatinine concentration is equal to that of serum. Treatment modalities of symptomatic lymphoceles encompass aspiration, sclerotherapy, drain placement, laparoscopic surgery, and open surgery. In the case of surgical intervention, marsupialization of the lymphocele and internal drainage in the peritoneal cavity is the method preferably employed [3, 5].

Urologic Complications

Urinary Leakage

Urinary leaks may develop within the first few days posttransplant or, in case of delayed graft function, at the onset of diuresis. They can occur at the level of the bladder, the ureter, or the renal pelvis, and may be caused by faulty handling of the donor ureter, ureteral slough secondary to disruption of ureteral blood supply, infection, hematoma, or rupture secondary to a tight ureteral stenosis. Clinical signs are, for example, decreased urine volume, severe pain, graft dysfunction, fever, wound drainage, and sepsis. A fluid collection can be diagnosed by ultrasound, should be drained under sterile conditions, and is typically accompanied by a high concentration of creatinine. In the event of urinary leakage, remedial action should be taken without delay. Placement of a urethral catheter can relieve the intravesical pressure and occasionally reduce or even stop the leakage altogether. Sometimes nephrostomy may be a suitable tool to diagnose the leak and to control the urine flow. Cases of ureteral leakage call for early surgical exploration, and usually necessitate reimplantation of the ureter into the bladder in an anti-refluxing fashion and placement of an indwelling double-J stent. If the ureter proves too short, the native ureter may be used to perform a ureteropyelostomy [3, 5, 23].

Urinary Tract Obstruction

Urinary tract obstruction is most often due to clots in the urinary tract, postoperative edema, or surgical complication. Ureteral obstruction is the most common urinary complication observed after kidney transplantation, which may happen early or late in the postoperative course. It is often due to technical problems or fibrosis and also may be due to BK polyomavirus infection or rejection episodes. Ureteral obstruction predominantly manifests itself as graft function deterioration without pain. Hydronephrosis can be shown by ultrasound, and hemodynamically relevant hydronephrosis by renal scintigraphy with furosemide. Depending on the site where the ureteral obstruction resides, a neoureterocystostomy, ureteropyelostomy, or nephrostoma may be necessary to perform [3, 5, 23].

Peritoneal Fluid Accumulation

After transplant, patients who were on peritoneal dialysis prior to transplant may have significant peritoneal fluid accumulation, likely due to peritoneal irritation from long-standing exposure to dextrose containing dialysate. Intermittent drainage of the abdomen via the peritoneal catheter may be warranted in the setting of patient discomfort or if there is concern for increased intra-abdominal pressure.

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Renal Transplantation: Immunosuppression and Managing Allograft Rejection

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Diagnosis and Management of Allograft Rejection 1945
Hyperacute Rejection 1945
Acute Rejection 1945
Chronic Rejection or Chronic Allograft Dysfunction 1951

Conclusions and Outlook 1952

References 1952

Abstract

During its lifetime, the renal allograft is at continuous risk for the development of rejection due to HLA and non-HLA incompatibility with the recipient which is why lifelong immunosuppressive therapy is required. The goal of immunosuppressive therapy posttransplantation in pediatric renal transplant recipients is to prevent acute and chronic rejection and support adherence while minimizing drug side effects and infectious complications. Most therapies alter immune response mechanisms but are not immunologically specific, and a careful balance is required to find the dose that prevents rejection of the graft, while minimizing the risks of over-immunosuppression leading to increased morbidity and mortality from infection and cancer. Although data from adult renal transplantation trials are used to help guide management decisions in pediatric patients, immunosuppressive therapy in pediatric renal transplant recipients is often modified due to the unique dosage requirements and clinical effects of these agents on the growth and development of children, who are less likely to have had prior exposure to pathogens. The optimal immunosuppressive therapy posttransplant has to be individualized to patient characteristics, and ideally should be titrated to the specific immune-risk thresholds of the recipient and to minimize any negative impact on growth. Evaluation of risk factors involves a comprehensive evaluation of clinical, biopsy, HLA mismatch, and viral findings, and the potential for recurrence of certain primary renal diseases such as lupus nephritis or focal segmental glomerular sclerosis. Allograft survival rates vary among the various immunosuppressive agents due to patient-specific clinical characteristics, such as age, obesity, ethnicity, hyperlipidemia, arterial hypertension, proteinuria and/or delayed allograft function. The "one size fits all" strategy needs to evolve into tailored strategies based upon a child's medical history and circumstances.

Keywords

Allograft rejection · Calcineurin inhibitors · Mycophenolate mofetil · Azathioprine · Everolimus · Glucocorticoids · Immunosuppressive induction therapy · Immunosuppressive maintenance therapy · Pediatric renal transplantation · Biomarkers

Introduction

The common immunosuppressive agents used in pediatric renal transplantation include antibodies to cell surface antigens on lymphocytes (anti-thymocyte globulin (ATG)), anti-interleukin 2 (IL-2) receptor antibodies, glucocorticoids, the calcineurin inhibitors tacrolimus and ciclosporin, the lymphocyte proliferation inhibitors mycophenolate mofetil (MMF) and azathioprine, and the mammalian target of rapamycin (mTOR) inhibitors sirolimus and everolimus. The optimal prophylactic induction immunosuppressive therapy remains controversial. Maintenance regimens consist of a combination of immunosuppressive agents that differ in their mechanism of action. This strategy minimizes morbidity and mortality associated with each class of agent while maximizing overall effectiveness. An adequate maintenance level of immunosuppression is required to dampen the immune response to the allograft. The level of chronic immunosuppression is slowly decreased over time to help lower the overall risk of infection and malignancy; these risks directly correlate with the degree of overall immunosuppression.

Allograft survival rates vary among the various immunosuppressive agents due to patient-specific clinical characteristics, such as age, obesity, ethnicity, hyperlipidemia arterial hypertension, proteinuria, delayed allograft function, and some donor-related factors such as living-related versus deceased kidney donation. Immunosuppressive agents should therefore be chosen in part based on patient characteristics. Other issues to be considered are related to the immunologic history of the patient, such as ABO compatibility and the degree of HLA mismatching, presensitization, retransplantation, history of acute rejection episodes, and the risk of recurrent disease.

Current immunosuppressive agents reduce acute rejection, but do not induce reliable or durable tolerance that can be accurately measured. A few patients with organ transplants successfully can withdraw their immunosuppression without rejecting their grafts for long periods of time, but the hunt for biomarkers to harness this “operational” tolerance state have remained elusive [1]. However, these are rare exceptions, and such patients may eventually reject, even after years as this operational state of tolerance is unstable and poorly understood. Much additional work is needed to define optimal immunosuppressive regimens in pediatric renal transplant recipients, particularly with respect to newer and evolving regimens, specifically their evaluation of safety in the immunologically naive pediatric population.

This chapter focuses on immunosuppressive therapy in pediatric renal transplant recipients and the diagnosis and management of allograft rejection.

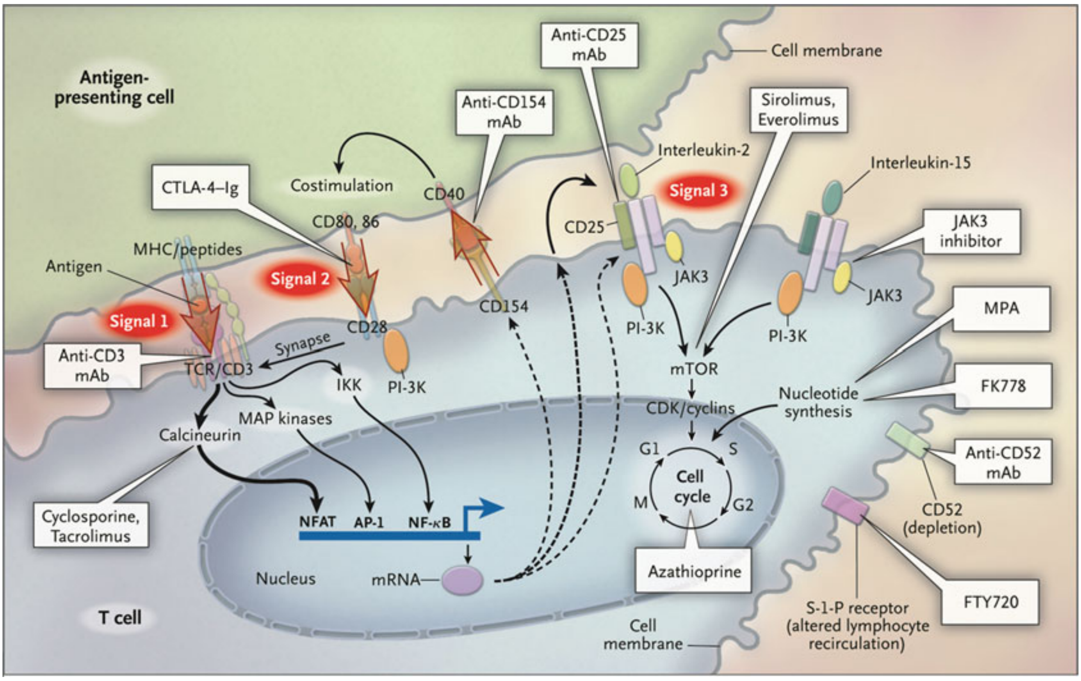
Classification of Immunosuppressive Agents

Immunosuppressive and immunomodulatory drugs can be pharmacologically categorized on the basis of their mechanism of action. The three-signal model of T cell activation and proliferation is helpful in understanding the molecular mechanisms and site of action of various immunosuppressive drugs. Figure 1 depicts a schematic representation of the three-signal model along with the site of action of common immunosuppressive drugs. Signal 1 features antigen-presenting cells (APCs; macrophages and dendritic cells) presenting the foreign antigen to the T lymphocyte, activating the T cell receptor (TCR), which further relays the signal through the transduction apparatus known as the CD3 complex. Signal 2 is a nonantigen-specific co-stimulatory signal which occurs as a result of binding of the B7 molecule on the APC to CD28 on the T cell. Both signal 1 and signal 2 activate signal transduction pathways: the calcium–calcineurin pathway, mitogen-activated protein (MAP) pathway, and the nuclear factor-κB (NF-κB) pathway. This in turn leads to increased expression of interleukin-2 (IL-2), which through its receptor (IL-2R) activates the cell cycle (signal 3). Signal 3 activation requires the enzyme target of rapamycin for translation of mRNA and cell proliferation. Thus, various drugs act on different cellular signals and achieve immunosuppression by a number of mechanisms: depleting lymphocytes, diverting lymphocyte traffic, or blocking lymphocyte response pathways.

Induction Immunosuppressive Therapy

Induction refers to the administration of an intensive immunosuppressive regimen during the perioperative period. The rationale behind this approach is that the risk of acute rejection is greatest in the first weeks or months posttransplant. Induction therapies often involve the use of polyclonal or monoclonal antibodies to achieve rapid and profound early immunosuppression. *Polyclonal antibodies* used for this purpose include those against thymocytes (anti-thymocyte globulin (ATG)); *monoclonal antibodies* include basiliximab (a chimeric human–murine anti-CD25 or anti–interleukin-2 receptor antibody), and alemtuzumab (an anti-CD52 antibody targeting both B and T cells), which is not always readily available.

Each protocol may have specific advantages and/or disadvantages in a particular patient population [3]. The optimal prophylactic induction


Antigen-presenting cell
Anti-CD25 mAb
Cell membrane
Anti-CD154 mAb
Sirolimus, Everolimus
Interleukin-2
Interleukin-15
CTLA-4–Ig
Costimulation
CD40
Signal 3
CD25
JAK3 inhibitor
CD80, 86
MHC/peptides
Antigen
Signal 2
JAK3
JAK3
CD154
MPA
Signal 1
CD28
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Anti-CD3 mAb
Synapse
TCR/CD3
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PI-3K
mTOR
Nucleotide synthesis
FK778
MAP kinases
CDK/cyclins
Calcineurin
G1
S
Anti-CD52 mAb
Cell cycle
M
G2
CD52 (depletion)
Cyclosporine, Tacrolimus
NFAT
AP-1
NF-κB
Nucleus
mRNA
Azathioprine
FTY720
S-1-P receptor (altered lymphocyte recirculation)
T cell
Cell membrane

Fig. 1 Individual Immunosuppressive Drugs and Sites of Action in the Three-Signal Model. Anti-CD154 antibody has been withdrawn from clinical trials but remains of interest. FTY720 engagement of sphingosine-1-phosphate (S-1-P) receptors triggers and internalizes the receptors and alters lymphocyte recirculation, causing lymphopenia. Antagonists of chemokine receptors (not shown) are also being developed in preclinical models. MPA denotes mycophenolic acid. (Modified according to [2])

immunosuppressive therapy to prevent renal transplant rejection remains therefore controversial. Figure 2 presents the induction antibody use from 2007–2018, as reported in the Scientific Registry of Renal Transplant Recipients (SRTR) 2018 annual report, where use of ATG has increased and that of anti-IL2R and no induction has decreased over time. In Europe, as reported by the Cooperative European Paediatric Renal Transplant Initiative (CERTAIN) Registry, 60% of pediatric renal transplant recipients do not receive any induction therapy, 35% receive basiliximab, and 5% ATG [4]. Hence, the frequency of use of the different immunosuppressive antilymphocyte regimens for induction therapy varies markedly between North America and Europe.

Induction therapy produces the greatest benefits in groups at high risk for allograft rejection. These high-risk groups include African Americans, recipients of kidneys with prolonged cold ischemia time, and those at high immunologic risk, particularly individuals who are pre-sensitized. The sequential induction regimen of thymoglobulin followed by a combination of tacrolimus and MMF with or without steroids is recommended in these high-risk groups.

The Polyclonal Lymphocyte-Depleting Antibody Thymoglobulin

Because of the redundancy of the immune system, polyclonal antibodies, which have a broad specificity, should theoretically be more effective in induction therapy than monoclonal antilymphocyte agents. *Thymoglobulin* is a rabbit-derived polyclonal antibody preparation approved for the treatment of rejection and recently also for induction therapy by the Federal Drug Administration (FDA) in USA. Thymoglobulin contains antibodies to a wide variety of T cell antigens and HLA antigens. Polyclonal antibodies act in three ways: By

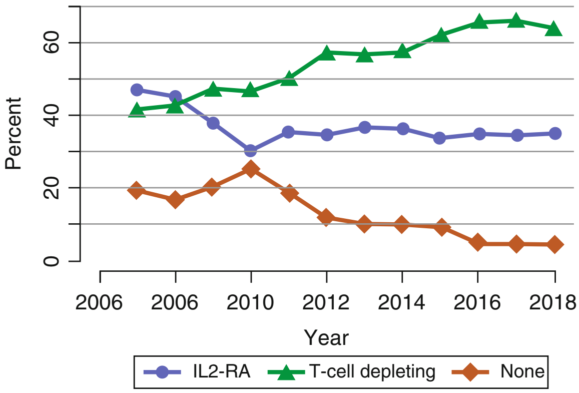

Fig. 2 Induction agent use in pediatric kidney transplant recipients in the United States of America. Immunosuppression at transplant reported to the Organ Procurement and Transplantation Network (OPTN) (https://pubmed.ncbi.nlm.nih.gov/31898417). IL2-RA, interleukin-2 receptor antagonist

activating or altering the function of lymphocytes, by lysing lymphoid cells, and by altering the traffic of lymphoid cells and sequestering them. These antibodies are potently immunosuppressive, but often produce side effects. By triggering T cells, they generate significant first-dose effects, with the release of tumor necrosis factor alpha (TNFα), interferon γ (IFN-γ), and other cytokines, causing a first-dose reaction (flu-like syndrome, fever, and chills).

Thymoglobulin induction is usually dosed from 1 to 6 mg/kg per dose, and the duration may range from 1 to 10 days, although a more typical regimen is 1.5 mg/kg per dose for 3–5 days [5–9]. The optimal total induction dose is felt to be 6 mg/kg [7, 10]. Higher doses and prolonged duration of induction agents are thought to be associated with an increased risk of infection and the potential development of lymphoma, whereas cumulative doses of less than 3 mg/kg may not effectively prevent acute rejection [10].

The Monoclonal IL-2 Receptor Antibody Basiliximab

Full T cell activation leads to the calcineurin-mediated stimulation of the transcription, translation, and secretion of IL-2, a key autocrine growth factor that induces T cell proliferation. It also leads to the de novo expression of CD25 on the T cell surface. Thus, an attractive therapeutic option is abrogation of IL-2 activity via the administration of anti-IL-2 receptor antibodies resulting in attenuated signaling via the high-affinity IL-2 receptor. The only anti-IL-2 receptor antibody currently available is basiliximab, a chimeric monoclonal antibody that has been approved by the FDA for use in renal transplantation in adults and pediatric patients. Basiliximab is directed against CD25, the IL-2 receptor 55 kDa α-chain. However, IL-2 receptor functions are partially redundant, because other cytokine receptors have overlapping functions, e.g., IL-15 receptors. Therefore, saturating IL-2 receptors produces stable, but relatively mild immunosuppression and is only effective in combination with other immunosuppressants. In contrast to thymoglobulin, basiliximab is not a lymphocyte-depleting antibody.

The dosing schedule for basiliximab is the following: Intravenous administration of two 10 mg doses to children <35 kg in weight and two 20 mg doses to children ≥35 kg in weight, with the first dose given peri-transplantation and the second on day four posttransplant. In patients on concomitant immunosuppression with ciclosporin and azathioprine, the mean duration of IL-2 receptor saturation is 42 ± 16 days [11], in patients on ciclosporin with MMF, MMF reduces basiliximab clearance and prolongs CD25 saturation from 5 to 10 weeks [12].

Two large prospective randomized controlled trials in pediatric renal transplant recipients showed that induction therapy with basiliximab in patients with low to standard immunological risk on maintenance therapy (tacrolimus in conjunction with azathioprine, or ciclosporin in conjunction with MMF) did not lead to a statistically significant reduction in the incidence of acute rejection episodes [13, 14]. In contrast, the

KDIGO clinical practice guidelines recommend induction therapy for all adult patients, with an IL-2 receptor blocker as first line for those not at high immunological risk [15]. There is some evidence favoring the use of IL-2 receptor blockers over no induction in adult renal transplantation [16]. However, it has been pointed out in the more recent literature that these studies mainly used outdated maintenance regimens [17]. No large randomized trial has examined the effect of IL-2 receptor antibodies or rabbit ATG induction versus no induction in patients receiving tacrolimus, mycophenolic acid, and corticosteroids. With this triple maintenance therapy, the addition of induction may achieve an absolute risk reduction for acute rejection of only 1–4% in standard-risk patients without improving renal allograft or patient survival. In contrast, rabbit ATG induction lowers the relative risk of acute rejection by almost 50% versus IL-2 receptor antibodies in patients with high immunological risk. These recent data raise questions about the need for IL-2 receptor antibodies in renal transplantation, as it may no longer be beneficial in standard-risk transplantation although augmentation of immunosuppression may allow corticosteroid minimization and may be inferior to rabbit ATG in high-risk situations. Updated evidence-based guidelines are necessary to support clinicians deciding whether and what induction therapy is required for their transplant patients today [17].

Alemtuzumab (Campath-1H, MabCampath) is a humanized IgG1 monoclonal antibody directed against CD52, a glycoprotein expressed on mononuclear cells, including T and B lymphocytes, monocytes, and natural killer cells [18]. Its efficacy for induction and maintenance with low-dose calcineurin inhibitors was first introduced by Calne et al. [19] and later supported by the Pittsburgh group [20] in adults and children. Alemtuzumab induction has been associated with lower rates of acute rejection than basiliximab and daclizumab in low immunological risk patients and was associated with similar efficacy as compared with rabbit anti-thymocyte globulin in high-risk patients [21], although most of these studies have involved adult renal transplant recipients. There is also pediatric experience that supports this efficacy claim, especially in highly sensitized, high-risk children [22, 23]. A significant reduction of white blood cell and absolute lymphocyte counts have been observed in children receiving alemtuzumab treatment up to 1 year posttransplant [24].

As a result, there is presently no consensus among pediatric renal transplantation centers regarding the use and regimen for immunosuppressive induction therapy, although there have been moves for standardization of immunosuppressive regimens for pediatric renal transplant recipients in some countries (e.g., United Kingdom). Considerations in choosing the appropriate agent include the efficacy in the patient population (e.g., recipients with high or low immunological risk of graft loss), the side effect profile, and the concomitant immunosuppressive therapy (corticosteroid avoidance, early corticosteroid withdrawal, or conventional corticosteroid therapy).

Maintenance Immunosuppressive Therapy

Maintenance immunosuppressive therapy is administered to renal transplant recipients to help prevent acute rejection. Although an adequate level of immunosuppression is required to dampen the immune response to the allograft, the level of chronic immunosuppression is slowly decreased over time to help lower the overall risk of infection and malignancy; these risks directly correlate with the degree of overall immunosuppression. The type of immunosuppression may also be varied to decrease the risk of developing chronic antibody-mediated rejection, the most common underlying long-term cause of renal allograft loss. Conventional maintenance regimens consist of a combination of immunosuppressive agents that differ in their mechanism of action. This strategy minimizes morbidity and mortality associated with each class of agent while maximizing overall effectiveness. Such regimens may vary by transplant center and geographic area.

There are a number of important issues to consider when deciding upon the immunosuppressive protocol to administer in a particular patient: The risk for acute rejection and allograft loss is highest in the first 3 months after transplantation. As a result, immunosuppression should be at its highest level during this period. The occurrence of the most serious side effects of immunosuppressive therapy, infections and malignancy, correlate with the total amount of immunosuppression. It is therefore essential that immunosuppression is gradually tapered to a maintenance level by 6–12 months posttransplant although reduction of immunosuppression may result in acute rejection and increased total burden of chronic immunosuppression as part of treatment.

Renal allograft survival rates vary among the various immunosuppressive agents due to patient-specific clinical characteristics, such as age, obesity, ethnicity, hyperlipidemia, arterial hypertension, proteinuria, and/or delayed allograft function. Immunosuppressive agents should therefore be chosen in part based on patient characteristics. Other issues to be considered are related to the “immunologic” history of the patient including degree of HLA mismatching, history of sensitization as determined by panel reactive antibodies, primary transplant or a re-transplant, history and number of acute rejection episodes in the current transplant, and the risk of recurrence of underlying kidney diseases such as focal segmental glomerulosclerosis (FSGS) and atypical hemolytic uremic syndrome (HUS).

The optimal maintenance immunosuppressive therapy in pediatric renal transplantation is not established. The major immunosuppressive agents currently used in various combination regimens are tacrolimus, ciclosporin, MMF, azathioprine, everolimus, sirolimus, and glucocorticoids (primarily oral prednisone or methylprednisolone). Most transplant centers currently utilize a maintenance regimen consisting of triple immunosuppression therapy with a calcineurin inhibitor (tacrolimus or ciclosporin), an anti-metabolite (MMF or azathioprine), and in some patients prednisone or methylprednisolone. Everolimus or sirolimus is also used by some transplant centers in triple therapy regimens, sometimes in place of the calcineurin inhibitor or the antimetabolite. Within the NAPRTCS registry, marked changes in the type of maintenance immunosuppression and dosing strategies have been observed over time [1]. These are substantially caused by the introduction of drugs such as MMF and tacrolimus (Table 1). In the European CERTAIN Registry in the transplant era 2010–2018, the most popular immunosuppressive regimen at posttransplant day 30, applied in 56% of patients, was triple therapy with tacrolimus, MMF, and prednisone [2]. According to the North American Scientific Registry of Transplant Recipients (SRTR) 2018 annual report,

Table 1 Observed drug utilization rates in North American pediatric renal transplant recipients among transplanted grafts with $\geq$30 days function that have occurred since 1996. (Data are from Ref. [25])

	Transplant era 1996–2001				Transplant era 2002–2007				Transplant era 2008–2017			
	30 days	1 year	3 year	5 year	30 days	1 year	3 year	5 year	30 days	1 year	3 year	5 year
Prednisone/CsA/MMF	35.4	38.1	30.6	22.4	9.7	8.6	7.9	7.5	1.7	1.9	0.5	0.7
Prednisone/CsA/Aza	23.1	17.7	14.2	8.9	0.8	0.8	0.6	0.7	0.1	0.2	0.3	0.4
Prednisone/CsA	10.7	4.4	3.8	3.5	1.5	0.8	0.3	0.8	0.4	0.3	0.2	0.0
Prednisone/TAC/MMF	14.3	19.6	24.4	30.1	51.3	49.6	44.2	42.1	48.9	41.7	38.6	33.1
Prednisone/TAC/Aza	2.3	4.9	6.5	6.9	1.7	2.4	2.7	3.9	2.0	2.3	4.3	6.7
Prednisone/TAC	4.2	5.0	6.7	6.9	4.1	5.8	6.7	6.2	2.9	8.2	8.0	6.7
TAC/MMF	0.4	1.1	1.7	2.5	10.7	9.4	11.5	13.1	33.8	27.3	26.5	27.5
Other combination	9.5	9.2	12.0	17.3	20.1	22.7	26.0	25.8	10.1	18.1	21.6	25.3

the most used maintenance immunosuppression in the USA is a combination of tacrolimus with MMF with or without steroids.

The Calcineurin Inhibitors Ciclosporin and Tacrolimus

Ciclosporin, a lipophilic cyclic peptide of 11 amino acid residues, and tacrolimus, a macrolide antibiotic, are drugs with similar mechanisms of action that have become major maintenance immunosuppressive agents used in transplantation. Ciclosporin and tacrolimus act by inhibiting the calcium-dependent serine phosphatase calcineurin, which normally is rate limiting in T cell activation.

Ciclosporin and tacrolimus are both variably absorbed and are metabolized extensively by the liver (via the cytochrome P450 system). Neither ciclosporin nor tacrolimus is affected by alterations in renal function. Both ciclosporin and tacrolimus bind to cells and to plasma components (primarily lipoproteins for ciclosporin and albumin for tacrolimus) in the blood; consequently, they must be assayed in whole blood. Many drugs and agents can affect ciclosporin and tacrolimus levels through effects on their absorption or metabolism (Table 2).

Table 2 Examples of common drug interactions of immunosuppressants used in solid-organ transplantation: Cyclosporine, tacrolimus, sirolimus, and everolimus

Common types of drug interactions	Examples of interacting drugs
Coadministration of drugs that inhibit CYP3A metabolism and/or P-gp efflux can increase immunosuppressant whole blood concentrations, leading to significant toxicities	Amiodarone ART-boosting agents (eg, ritonavir, cobicistat) Azole antifungals (eg, fluconazole, posaconazole, voriconazole) HIV protease inhibitors (eg, atazanavir, nelfinavir, saquinavir) Macrolide antibiotics (except azithromycin) Non-dihydropyridine calcium channel blockers Ombitasvir-paritaprevir-ritonavir with or without dasabuvir (an HCV, direct-acting antiviral regimen) Grapefruit juice
Coadministration of drugs that induce CYP3A metabolism and/or P-gp efflux pumping can decrease immunosuppressant whole blood concentrations, increasing the risk of organ rejection	Antiseizure drugs, enzyme inducing (eg, carbamazepine, fosphenytoin, oxcarbazepine, phenobarbital, phenytoin, primidone) Enzalutamide Nafcillin Rifamycins (eg, rifabutin, rifampin, rifapentine) St. John's wort
Coadministration of nephrotoxic drugs with cyclosporine or tacrolimus can cause additive or synergistic kidney injury	Aminoglycosides Amphotericin B Colchicine Nonsteroidal antiinflammatory drugs (NSAIDs)
Coadministration of drugs that increase serum potassium with cyclosporine or tacrolimus may cause severe hyperkalemia	ACE inhibitors/ARBs Amiloride Spironolactone Triamterene Trimethoprim, trimethoprim-sulfamethoxazole (cotrimoxazole)
Coadministration of statin drugs with cyclosporine can increase statin levels and risk of myotoxicity	Atorvastatin Lovastatin Pitavastatin Rosuvastatin Simvastatin

CYP, cytochrome P450 metabolism; P-gp, P-glycoprotein drug efflux pump; ART, HIV antiretroviral therapy; HIV, human immunodeficiency virus; HCV, hepatitis C virus; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker

Since the absorption of ciclosporin is decreased and its metabolism increased in children compared to adults, relatively higher dosages are required in pediatric patients. Ciclosporin is usually administered initially as 8–15 mg/kg per day in divided doses (or intravenously using one-third the oral dose over a 24-h period) during the induction phase, with target trough blood levels of 150–300 μg/L for the first 3–6 months posttransplant. Doses are reduced after 3–6 months (typically 4–6 mg/kg daily); long-term target trough blood levels of 75–125 μg/L appear to provide comparable patient and graft survival as higher blood levels but with less risk of malignancy [26]. Generic forms of ciclosporin are available, but they may not be equivalent and readily interchangeable, and knowledge of the characteristics of the oral formulations is necessary before switching between them.

Tacrolimus is 20- to 30-fold more potent than ciclosporin and, therefore, is administered at a 20-fold lower dose. Initial dosing is usually 0.2–0.3 mg/kg per day in two divided doses orally (or 0.05–0.1 mg/kg per day intravenously over 24 h), and target trough levels are 5–15 μg/L. Since tacrolimus is more water soluble than ciclosporin, it is not as dependent upon bile salts for absorption. However, food intake can reduce the absorption of tacrolimus by up to 40%; thus, it is recommended that this agent be taken on an empty stomach [27] or at least patient takes medication the same time every day. In addition, tacrolimus is best absorbed in the morning. There is also an extended-release formulation of tacrolimus available that is designed to be given once per day with similar efficacy and safety.

Genetic polymorphisms in genes encoding tacrolimus-metabolizing enzymes partly explain the interpatient variability in tacrolimus pharmacokinetics [28]. The key enzymes involved in the metabolism of tacrolimus are CYP3A4 and CYP3A5 [29]. Individuals are considered expressers of CYP3A5 if they carry at least one CYP3A5*1 allele, whereas individuals homozygous for the CYP3A5*3 allele are classified as CYP3A5 nonexpressers. In addition to CYP3A5*3, the CYP3A5*6 and CYP3A5*7 variant alleles can also lead to nonfunctional CYP3A5 protein [30]. There are also ethnic distribution differences of CYP3A5 variant alleles with expressers (carriers of the CYP3A5*1 variant allele) being more frequently found among non-Caucasian populations. Approximately 10–40% of Caucasians are CYP3A5 expressers, 33% of Asians and 55% of African Americans [31]. CYP3A5 expressers require a tacrolimus dose that is approximately 1.5-fold to twofold higher than nonexpressers to reach the same exposure [32, 33]. This implies that following a standard, body weight–based tacrolimus dose, CYP3A5 expressers are prone to have subtherapeutic tacrolimus concentrations whereas nonexpressers are expected to have supratherapeutic tacrolimus concentrations [34].

The overall conclusion from data in adult renal transplant recipients is that tacrolimus-based immunosuppression is associated with decreased acute rejection rates, a superior long-term renal allograft function and more favorable cardiovascular risk profile than ciclosporin microemulsion–based immunosuppression which translates into improved long-term renal allograft survival. In pediatric patients, the efficacy and safety of tacrolimus and ciclosporin were compared in one multicenter trial in 196 patients, who were randomly assigned to receive either tacrolimus or ciclosporin microemulsion administered concomitantly with azathioprine and corticosteroids [35]. Tacrolimus therapy resulted in a significantly lower incidence of acute rejection (36.9%) compared with ciclosporin therapy (59.1%) ($p = 0.003$). The incidence of steroid-resistant rejection was also significantly lower in the tacrolimus group compared with the ciclosporin group (7.8% versus 25.8%, $p = 0.001$). The difference was also significant for biopsy-confirmed acute rejection (16.5% versus 39.8%, $p < 0.001$). Patient survival at 1 year was similar (96.1% versus 96.6%); ten grafts were lost in the tacrolimus group compared with 17 graft losses in the ciclosporin group ($p = 0.06$). The tacrolimus group had a significantly better estimated GFR at 1 year. A follow-up study at 4 years showed that patient survival was similar (94% versus 92%, $p = 0.86$), but renal allograft survival (86% versus 69%; $p = 0.025$) and mean estimated glomerular filtration rate significantly favored tacrolimus [36]. Cholesterol remained significantly higher with ciclosporin throughout follow-up. Three patients in each arm developed posttransplant lymphoproliferative disorder (PTLD). Incidence of new onset of diabetes mellitus after transplantation was not different. Therefore, tacrolimus is significantly more effective than ciclosporin in preventing acute rejection in pediatric renal recipients, and renal function and graft survival are also superior with tacrolimus.

Ciclosporin and tacrolimus have similarities and differences in their *toxicity profiles* (Table 3). Both can cause nephrotoxicity, hyperkalemia, hyperuricemia with occasional gout, hypomagnesemia secondary to urinary loss, arterial hypertension, diabetes mellitus, and neurotoxicity, especially tremor. In the European pediatric study, the incidence of hypomagnesaemia was significantly higher in the tacrolimus-treated group (34%) compared with the ciclosporin-treated group (12.9%) [35]. Similarly, diarrhea was more frequent in tacrolimus-treated patients (13.6% versus 3.2%). Hypertrichosis, gingival hyperplasia, and flu-like syndrome were reported only in ciclosporin-treated patients and tremor was reported only in tacrolimus-treated patients [35]. Those results are similar to adults where tremor is consistently more common with tacrolimus and hirsutism and gum disease more common in ciclosporin-treated patients [37]. Arterial hypertension and hyperlipidemia are more commonly observed with ciclosporin. In the European pediatric study, the mean total cholesterol levels were reported to decrease in the tacrolimus group and increase in the ciclosporin group at the end of 6 months [24]. In the multicenter analysis from the European CERTAIN Registry, the prevalence of dyslipidemia was 95% before transplantation and 88% at 1-year posttransplant [38]; the use of tacrolimus and of MMF was associated with significantly lower concentrations of all lipid parameters compared to regimens containing ciclosporin and mTOR inhibitors. Regimens consisting of ciclosporin, MPA, and corticosteroids as well as of ciclosporin, mTOR inhibitors, and corticosteroids

Table 3 Semi-quantitative comparison of safety profiles of current primary immunosuppressive compounds

	Tacrolimus	Cyclosporine	Mycophenolate mofetil	Sirolimus/ Everolimus	Glucocorticoids
Nephrotoxicity[a]	++(+)	+++	–	+	–
Hyperlipidaemia	+(+)	++	–	+++	++
Arterial hypertension	++	+++	–	–	+
Neurotoxicity	+++	+++	–	–	+
Posttransplant diabetes mellitus	+++	++	–	–	++
Bone marrow suppression	–	–	++	++	–
Gastrointestinal adverse effects[b]	+	+	+++	+	–
Hepatotoxicity	+	+	–	+	–
Esthetical changes	+	++	–	–	++
Wound healing problems[c]	–	–	+	++	+
Pulmonary toxicity	–	–	–	+	–
Fetal toxicity	+	+	++	NA	–
Osteoporosis	+	+	–	?	++
Inhibition of longitudinal growth	–	–	–	+	+++

– indicates the drug has no effect on this adverse effect; **+** indicates mild; **++** indicates moderate; **+++** indicates severe; **?** indicates clinical data available but insufficient to provide conclusions; NA = no information available

[a] Sirolimus without calcineurin inhibitor

[b] Gastrointestinal disorders: diarrhoea, abdominal pain, nausea and vomiting, ileus, rectal disorders, mucosal ulcerations

[c] Wound healing problems including lymphocele formation

were associated with a threefold and 25-fold increased risk of having more than one pathologic lipid parameter as compared to the use of tacrolimus, MMF, and corticosteroids [38]. Similarly, in adults, several studies have shown that lipid levels are much lower in tacrolimus-treated patients than in those receiving ciclosporin [37]. The improved lipid profiles on tacrolimus may contribute to a better long-term outcome with less cardiovascular morbidity.

On the other hand, tremor and glucose intolerance are more common with tacrolimus. In the pediatric multicenter European study there was, however, no difference in the incidence of new-onset insulin-dependent diabetes mellitus between tacrolimus- (3%) and ciclosporin-treated patients (2.2%) [35, 36]. The incidence of diabetes mellitus with tacrolimus immunosuppression has become less frequent in recent randomized trials comparing these two calcineurin inhibitors [39]. Posttransplant diabetes mellitus can regress after dose reduction in some but not all patients. Both reduction of corticosteroid dosage and a low target trough tacrolimus concentration contribute to the recent marked reduction of the incidence of diabetes mellitus under tacrolimus immunosuppression in both adults and children [35, 37]. Ciclosporin may also be associated with coarsening of facial features, especially in children. Bone pain that is responsive to calcium channel inhibitors may also occur with ciclosporin or tacrolimus use [40].

The most common serious problem with the calcineurin inhibitors is *nephrotoxicity*, with both a reversible vasomotor component and an irreversible component. Both ciclosporin and tacrolimus can cause acute elevations in serum creatinine that reverse with reduction of the dose, apparently caused by renal vasoconstriction which itself may be mediated by calcineurin inhibition. Chronically, ciclosporin and tacrolimus can induce tubular atrophy and interstitial fibrosis with characteristic hyalinosis of the afferent arteriole [41]. The importance of this lesion is apparent from studies in cardiac and liver transplant recipients, in whom ciclosporin or tacrolimus use is associated with chronic kidney disease progressing to end-stage kidney disease in a significant fraction of patients [42]. This problem was more acute at a time, when higher doses of ciclosporin were administered for longer periods. Currently, ciclosporin and tacrolimus toxicity are associated with only mild to moderate declines in renal function. However, as the number of patients with long-standing non--renal transplants increases, there is increasing concern about future end-stage kidney disease in this population. It is important to establish the diagnosis of calcineurin inhibitor nephrotoxicity by percutaneous renal biopsy and reduce or stop calcineurin inhibition whenever possible [39, 43, 44].

There is no difference in the incidence of PTLD between tacrolimus- and ciclosporin-microemulsion-treated recipients when used in combination with azathioprine and corticosteroids [1% (1/103) versus 2.1% (2/93)] [35] or when used in conjunction with MMF/corticosteroids (1.4% versus 2%) [45]. This is similar to adults, in whom recent large, randomized studies could not show any difference in the incidence of malignancy between patients treated with tacrolimus or ciclosporin [37].

Therapeutic Drug Monitoring

Tacrolimus and ciclosporin are drugs with a *narrow therapeutic index* and a broad intraindividual and interindividual pharmacokinetic variability. Serious clinical consequences may occur because of under- or overdosing. Hence, individualization of calcineurin inhibitor dosage by therapeutic drug monitoring is required. When tacrolimus is utilized, a monitoring strategy based on trough levels is in general sufficient, because trough levels are good indicators of systemic exposure. In most transplant centers, doses are adjusted to attain target whole-blood trough concentrations of 8–12 μg/L during the first 3 months after transplantation, between 5 and 10 μg/L during month 4–12, and 4–8 μg/L thereafter. It must be emphasized that these target ranges are dependent on the concomitant immunosuppressive therapy. In the SYMPHONY trial, for example, low tacrolimus exposure (trough levels between 3 and 7 μg/L) in the first-year posttransplant in conjunction with MMF, prednisone, and daclizumab induction was associated with excellent efficacy and little tacrolimus-associated toxicity [46]. Therapeutic drug monitoring is required for individualization of dosage of ciclosporin. The traditional monitoring strategy for ciclosporin is based on pre-dose trough level measurements (C_0). One aims for the following trough levels in conjunction with MMF therapy and prednisone: Months 0 to 3 posttransplant: 120–200 μg/L, thereafter 80–160 μg/L. With the microemulsion formulation, ciclosporin levels at 2 h post-dose (C_2) have been found to correlate better with total drug exposure and immunosuppressive efficacy than C_0 levels [47–49]. A C_2 blood sample also has a higher proportion of parent drug when compared with a C_0 sample; this results in better agreement in C_2 levels measured by different assay methodologies than occurs with C_0 concentrations, which have a greater proportion of metabolites. However, error margins are greater for C_2 levels if the sample is not collected within a narrow (± 15 min) time window. Overall, C_2 monitoring has not been universally adopted.

Antiproliferative Agents

Mycophenolate Mofetil

Mycophenolate mofetil (MMF) impairs lymphocyte function by blocking purine biosynthesis via inhibition of the enzyme inosine monophosphate dehydrogenase (IMPDH). MMF was developed as a replacement for azathioprine for maintenance immunosuppression. It is not nephrotoxic, and has less bone marrow toxicity than azathioprine. However, gastrointestinal toxicity can occur, usually manifested by gastritis and diarrhea. Mycophenolic acid (MPA), the active ingredient of the prodrug MMF, acts by blocking de novo purine synthesis in lymphocytes. Purines can be generated by de novo synthesis or by recycling (salvage pathway), and lymphocytes preferentially use the former. MPA is a selective and noncompetitive inhibitor of inosine monophosphate dehydrogenase 2 (IMPDH2), the rate-limiting enzyme in the de novo purine biosynthetic pathway for converting inosine monophosphate to guanosine monophosphate (GMP). Depletion of GMP by MPA has a relatively selective anti-proliferative effect on lymphocytes. In vitro, MMF blocks the proliferation of T and B cells, and inhibits clonal expansion. This results in inhibition of antibody production, generation of cytotoxic T cells, and development of delayed-type hypersensitivity. MPA is a more selective inhibitor of T- and B-cell proliferation than azathioprine due to greater dependence of lymphocytes on de novo synthesis of purines as compared to the "salvage" pathway. Furthermore, MPA impairs the ability of dendritic cells to present antigen, suppresses the recruitment of monocyte lineage cells, suppresses the glycosylation of adhesion molecules, inhibits vascular smooth muscle proliferation, improves endothelial function, and inhibits mononuclear cell recruitment into allografts and nephritic kidneys [50]. MPA also decreases cytokine-induced nitric oxide synthesis and prevents the formation of reactive species such as peroxynitrite. Furthermore, MPA exhibits antioxidant effects in experimental nephropathies. These properties of MPA likely augment its immunosuppressive properties by limiting fibrosis and vascular sclerosis after immunological injury [51].

MMF, a semisynthetic ethyl ester of MPA, is rapidly and completely absorbed and hydrolyzed by esterases to yield the active drug MPA. The *recommended dose* in pediatric patients in conjunction with ciclosporin is 1200 mg/m^2 per day in two divided doses, the recommended MMF dose in conjunction with tacrolimus is 600–800 mg/m^2 per day in two divided doses. However, data from a large prospective randomized study in both pediatric and adult renal transplant recipients on fixed dose MMF versus a concentration-controlled regimen, the FDCC study, indicate that a higher initial MMF dose, for example, 1800 mg MMF/m^2 per day in conjunction with ciclosporin and 1200 mg MMF/m^2 per day in conjunction with tacrolimus for the first 2–4 weeks posttransplant, is required to achieve adequate MPA exposure in the majority of patients [52, 53]. The MMF dose should be reduced with active CMV infection. When MMF is associated with diarrhea (a side effect of MMF, see below), dividing up the daily dosing to three to four doses per day may be effective in controlling the diarrhea.

The difference in MMF dosing depending on the concomitant calcineurin inhibitor is explained by a pharmacokinetic interaction of ciclosporin with the main MPA metabolite 7-O-MPA glucuronide (7-O-MPAG). Ciclosporin inhibits the multidrug resistance protein 2-mediated transport of 7-O-MPAG into the bile. MPAG is subject to enzymatic and nonenzymatic hydrolysis in bile and more importantly in the intestine, thereby liberating the unconjugated drug MPA, which is then reabsorbed into the systemic circulation. This enterohepatic circulation is responsible for a secondary MPA peak occurring six to 12 h after administration. The impact of the enterohepatic cycle on the MPA plasma concentration varies within and between individuals due to factors such as meal times or co-medication of drugs that interrupt the enterohepatic circulation (e.g., bile acid sequestrants and antibiotics). These factors should be considered when evaluating MPA concentrations (particularly pre-dose concentrations) in clinical practice. Furthermore, genetic abnormalities and disease can affect enterohepatic cycling and thus the bioavailability of MPA [54].

The metabolism of MPA due to glucuronidation can also be affected by drug induction. Glucocorticoids are known inducers of UDP-glucuronosyltransferases in vitro. When corticosteroids were completely withdrawn 12 months after transplantation, a 33% increase in the mean dose–normalized MPA pre-dose concentrations and MPA-AUCs was observed compared with concentrations at 6 months, when the patients were still receiving maintenance doses of steroids [55].

Following the success of the early MMF studies in adults, MMF was investigated in pediatric renal transplant recipients in open-label studies with historical controls. Data from three large multicenter studies [56–60] and one smaller study [61] provided support for the *safety and efficacy of MMF* in the pediatric renal transplant population when used with ciclosporin and prednisone. The incidence of acute rejection within the first 6–12 months for patients receiving MMF in these studies ranged from 28% to 37% [56, 58, 60]. Those studies comparing MMF patient groups to historical controls reported significant reductions in the incidence of acute rejection with MMF versus AZA [58, 60]. There was also a significant improvement in the incidence of acute rejection between patients receiving MMF and those receiving azathioprine at 3 years in a follow-up report to one study [59]. In one large study [56, 57], there were no differences in the incidence of acute rejection when the results were stratified by age. Medium-term (3-year) renal allograft and patient survival were excellent, with a 30% incidence of acute rejection [57]. MMF has a role in the prevention and/or treatment of chronic rejection. Among children with chronic rejection, some evidence suggests that substituting MMF for azathioprine may improve renal function [62, 63]. On the other hand, very long-term differences between MMF and azathioprine as anti-proliferative agents in at least in adult renal transplantation may be negligible [64], perhaps because azathioprine requires only once-daily dosing, which has been associated with improved adherence in particular in the setting of transition to adult care [65].

The major *toxicity of MMF* is gastrointestinal, mainly diarrhea, possibly as a result of the high concentrations of acyl-MPAG in the gut. In the MMF suspension trial in pediatric renal transplant recipients, safety of MMF was evaluated based on the occurrence of adverse events, including the development of opportunistic infections and malignancies. The most frequently noted adverse events were hematological problems such as leukopenia and gastrointestinal disorders such as diarrhea, which occurred in 25% and 16% of all patients, respectively, and were observed more often in the youngest age group. In general, the risk of developing side effects declines with increasing age. MMF is devoid of intrinsic renal, cardiovascular, or metabolic toxicities, but can increase the risk for CMV infections, leukopenia, and mild anemia (Table 3). MMF should not be used in pregnant transplant patients.

Patients on standard-dose MMF therapy show considerable between-patient variability in pharmacokinetic parameters. This variability is attributable to factors that influence exposure to MMF, such as renal allograft function, serum albumin levels, and use of concomitant medications such as ciclosporin that inhibit enterohepatic recirculation of the active metabolite of MMF, MPA, and genetic polymorphisms of MPA-metabolizing enzymes [54]. This variability is clinically relevant, as higher plasma concentrations of MPA are correlated with reduced risk of acute rejection after renal transplantation [66–68]. These findings have suggested that individualizing the dose regimen of MMF may further improve clinical outcomes compared with a standard-dose regimen.

There has been considerable debate regarding the utility of measuring MPA levels. Advocation for *MPA monitoring* is based on the premise that monitoring will result in avoiding both underdosing, which prevents rejection, and overdosing, which increases the risk of adverse reactions [67, 68]. One prospective randomized study in adults, for example, showed significantly fewer treatment failures and acute rejection episodes in the monitoring arm compared with the fixed-dose arm with no significant difference in side effects [69]. Within this study, MPA exposure and MMF dosing were higher in the monitoring arm based on three levels measured over the first 3 h post-dose (limited sampling strategy). Therefore, some transplant centers monitor MPA levels and target pre-dose levels between 1.5 and 4 mg/L. Some centers monitor MPA levels also as a measure of adherence to the immunosuppression plan. In general, monitoring of MPA exposure by MPA pre-dose plasma levels is more popular in clinical practice than monitoring of the area under the concentration time curve (MPA-AUC), for example, by a *limited sampling strategy*, but less precise. An MPA-AUC > 40 mg × L/12 h has been recommended for sufficient MPA exposure for the prevention of acute rejection episodes [68]. However, AUC monitoring, while being more accurate, is also more cumbersome and expensive.

Azathioprine

Azathioprine is a purine analogue derived from 6-mercaptopurine (6-MP), which has been widely used in renal transplantation for four decades, but is now substituted by the more efficacious MMF in most transplant centers. Azathioprine is metabolized in the liver to 6-MP and further converted to the active metabolite thioinosinic acid (TIMP) by the enzyme hypoxanthine–guanine phosphoribosyltransferase. Some but not all the immunosuppressive activity of azathioprine is attributable to 6-MP. Azathioprine acts mainly as an antiproliferative agent by interfering with normal purine pathways, by inhibiting DNA synthesis, and by being incorporated itself into DNA, thereby affecting the synthesis of DNA and RNA [70]. By inhibiting the synthesis of DNA and RNA, azathioprine suppresses the proliferation of activated B and T lymphocytes. In addition, azathioprine has been shown to reduce the number of circulating monocytes by arresting the cell cycle of promyelocytes in the bone marrow.

Thiopurine methyltransferase (TPMT) enzyme activity is a major factor determining azathioprine and 6-MP metabolism, and therefore 6-thioguanine (6-TG) and 6-methylmercaptopurine (6-MMP) levels. Approximately 89% of the population has wild-type TPMT, which is associated with normal or “high” TPMT enzyme activity, while 11% are heterozygous and have corresponding low TPMT enzyme activity. Most importantly, 0.3% of the population is homozygous for mutations of TPMT and thus have negligible activity, which causes 6-MP to be preferentially metabolized to produce high levels of 6-TG, which then leads to bone marrow suppression. Intermediate and normal metabolizers can have up to a threefold difference in initial target doses of azathioprine and 6-MP to achieve therapeutic 6-TG concentrations. We suggest obtaining both thiopurine methyltransferase (TPMT) genotype and TPMT enzyme activity (phenotype) testing in all patients who are evaluated for treatment with azathioprine or 6-mercaptopurine (6-MP). Genotype testing is suggested by the manufacturer and the US Food and Drug Administration.

Azathioprine is administered orally at 1.5 mg/kg per day (45 mg/m^2 body surface area per day) in conjunction with calcineurin inhibitors and 2.5 mg/kg per day, when used without calcineurin inhibitors. It is metabolized in the liver to 6-MP and further converted to the active metabolite TIMP. As 6-MP is degraded by xanthine oxidase, allopurinol (a xanthine oxidase inhibitor) will increase the levels of TIMP. Severe leukopenia can occur if allopurinol (used for the treatment of hyperuricemia and gout) is given with azathioprine, so allopurinol should generally be avoided in patients treated with azathioprine. The major side effect of azathioprine is bone marrow suppression. All three hematopoietic cell lines can be affected, leading to anemia, leukopenia, and thrombocytopenia. The hematologic side effects are dose related and can occur late in the course of therapy. They are usually reversible upon dose reduction or temporary discontinuation of the drug. Azathioprine should be temporarily withheld if the white cell count falls below 3000/mm^3 or if the count drops by 50% in consecutive checks. Recovery usually occurs within 1–2 weeks and then azathioprine can be restarted at a lower dose and increased gradually to the usual maintenance dose while monitoring the white cell count. Hepatotoxicity is another potentially serious side effect of azathioprine, which requires decreasing the dose or even discontinuation. Azathioprine has also been linked to the development of skin cancer, the most common malignancy in renal transplant recipients. As a result, patients taking azathioprine for a prolonged period should be instructed to avoid direct exposure to sunlight or to use heavy sun screens when exposed. However, other immunosuppressants also contribute to the increased skin cancer risk in transplant recipients [71, 72]. The recommendations to minimize sun exposure are therefore not azathioprine specific. Other side effects of azathioprine include increased susceptibility to infection and hair loss.

The Target of Rapamycin (TOR) Inhibitors Everolimus and Sirolimus

Sirolimus (rapamycin) is a macrocyclic triene antibiotic that is produced by the actinomycete *Streptomyces hygroscopicus*. Sirolimus was approved in September 1999 by FDA and in December 2000 by the European Medicines Agency for use in adult renal transplant recipients. Everolimus is an analogue of sirolimus that has similar effectiveness and side effect profile as sirolimus, but different pharmacokinetics (shorter half-life). Everolimus and sirolimus are both macrolide derivatives and share many pharmacokinetic features, including a close correlation between total exposure and trough concentration, low absorption that varies between and within patients, and differences in absorption between adults and children [73, 74]. Everolimus is the 40-O-(2-hydroxyethyl) derivative of sirolimus, a modification that results in some important pharmacokinetic differences between the two drugs. Everolimus is more hydrophilic than sirolimus and is absorbed more rapidly from the gut with more systemic clearance than sirolimus [75]. As a result, the elimination half-life of everolimus is shorter than for sirolimus (mean 28 h versus 62 h) [76, 77]. The clinical effect is that no loading dose is required for everolimus and that it is administered twice a day both in adults in children. There are more data available on the use of everolimus than sirolimus in pediatric renal transplantation.

Everolimus displays a novel mechanism of immunosuppressive action, which is distinct from that of the other immunosuppressive drugs. Everolimus first binds to the cytosolic immunophilin FK-binding protein 12 (FKBP12). This complex binds to and inhibits the activation of the mammalian target of rapamycin (mTOR), a key regulatory kinase. This inhibition suppresses cytokine-mediated T cell proliferation inhibiting the progression from the G1 to the S phase of the cell cycle. Thus, everolimus acts at a later stage in the cell cycle than do the calcineurin inhibitors ciclosporin and tacrolimus. Everolimus can, therefore, be used in combination with the calcineurin inhibitors to produce a synergistic effect [78].

Everolimus is available as tablets and dispersible tablets for administration in water. The current evidence from pediatric renal transplantation suggests that everolimus be administered at an initial dose of 0.8 mg/m^2 body surface area twice daily when given in combination with ciclosporin therapy, adjusted to target a trough concentration of 3–8 μg/L [79, 80]. There is a well-documented drug-drug interaction between mTOR inhibitors and ciclosporin [74], arising from a shared metabolic pathway (via the cytochrome P450 CYP3A4 isoenzyme system) and the fact that both are substrates for the drug transporter P-glycoprotein. Everolimus exposure is increased by up to threefold in patients receiving concomitant ciclosporin

[81, 82], while tacrolimus exerts only a minimal effect [82, 83]. In patients receiving everolimus with concomitant tacrolimus, a dose of 2 mg/m^2 body surface area twice daily is therefore appropriate [81].

The most frequent reason to include an mTOR inhibitor in the immunosuppressive regimen is to facilitate a reduction in calcineurin inhibitor exposure, or to eliminate calcineurin inhibitor therapy entirely. The current evidence suggests that de novo administration of everolimus with low-exposure calcineurin inhibitor therapy in children undergoing renal transplantation is efficacious and safe. The recently published 36-month, multicenter, open-label randomized study investigated everolimus with reduced-dose tacrolimus and corticosteroid elimination from month 5 posttransplant compared with a standard-dose tacrolimus regimen with MMF and corticosteroids (control) [84]. The incidence of composite efficacy failure (biopsy-proven acute rejection [BPAR], renal allograft loss, or death) at month 36 was 9.8% versus 9.6% for everolimus with reduced-dose tacrolimus and MMF with standard-dose tacrolimus, respectively, which was driven by BPARs. Renal allograft loss was low (2.1% versus 3.8%) with no deaths. Mean estimated glomerular filtration rate at month 36 was comparable between groups. Growth in pre-pubertal patients on everolimus with reduced-dose tacrolimus without glucocorticoids was better ($p = 0.05$) versus MMF with standard-dose tacrolimus and glucocorticoids. The overall incidence of adverse events and serious adverse events was comparable between groups. Rejection was the leading adverse event for study drug discontinuation in the everolimus with reduced-dose tacrolimus group. The authors concluded that, although adverse events–related study drug discontinuation was higher, an everolimus with reduced-dose tacrolimus regimen represents an alternative treatment option that enables withdrawal of corticosteroids as well as reduction of calcineurin inhibitors for pediatric renal transplant recipients [84].

Use of de novo everolimus with complete calcineurin inhibitor avoidance has not been explored in large trials in pediatric transplant recipients, but is unlikely to be preferable to concomitant reduced-exposure calcineurin inhibitor. Switching maintenance patients to an mTOR inhibitor to facilitate calcineurin inhibitor minimization can improve renal allograft function or avoid further functional deterioration, particularly when undertaken before irreversible damage has developed. Late switch below an eGFR of 40 mL/min/1.73 m^2, however, may be associated with an increase in preexisting proteinuria due to podocytopenia, favoring early conversion. It remains unresolved whether calcineurin inhibitor therapy should be reduced or, indeed, eliminated in maintenance patients regardless of whether renal dysfunction is believed to be due to calcineurin inhibitor–related nephrotoxicity. Currently, many transplant centers use mTOR inhibitors as part of a maintenance immunosuppressive regimen only in the following patient subsets in which this drug class may have particular utility: (i) In patients who have histologically proven calcineurin inhibitor nephrotoxicity despite low levels and doses of the calcineurin inhibitor; (ii) in patients with malignancy (e.g., skin cancers and Kaposi sarcoma), either in remission or being actively treated; (iii) after treatment of B cell PTLD; and (iv) in patients with recurrent CMV viremia, because everolimus has anti-CMV activity in vitro and is associated with less CMV replication and disease in vivo compared to MMF [85, 86]. Notably, the incidence of Epstein-Barr virus (EBV) or BK polyomavirus infection is not lower in everolimus- compared to MMF-treated patients [84].

Clinically relevant *adverse effects* of everolimus that require a specific therapeutic response or can potentially influence short- and long-term patient morbidity and mortality as well as renal allograft survival include hypercholesterolemia, hypertriglyceridemia, infectious and noninfectious pneumonia, anemia, lymphocele formation, and impaired wound healing (Table 3). There continues to be concern regarding male fertility in the setting of mTOR inhibition [87], and primarily mTOR-based, calcineurin inhibitor–free immunosuppression is associated with a significantly increased risk for the development of donor-specific antibodies [88]. These drug-related adverse effects are important determinants in the choice of a tailor-made immunosuppressive drug regimen that complies with the individual patient risk profile. Equally important in the latter decision is the lack of severe intrinsic nephrotoxicity associated with everolimus and its advantageous effects on arterial hypertension, posttransplantation diabetes mellitus, and esthetic changes induced by calcineurin inhibitors. Mild and transient thrombocytopenia, leukopenia, gastrointestinal adverse effects, and mucosal ulcerations are all minor complications of everolimus therapy that have less impact on the decision for choosing this drug as the basis for tailor-made immunosuppressive therapy.

Glucocorticoids

Glucocorticoids, developed in the early 1950s, represent one of the principal agents used for both maintenance immunosuppression and treatment of acute rejection. Glucocorticoids have both anti-inflammatory and immunosuppressive actions [89]. Lymphopenia and monocytopenia occur with the inhibition of lymphocyte proliferation, survival, activation, homing, and effector functions. The major glucocorticoids used are prednisone or prednisolone (given orally with comparable efficacy) and methylprednisolone (given orally or intravenously with 25% more potency). These agents are rapidly absorbed and have short plasma half-lives (60–180 min), but long biological half-lives (18–36 h).

In many transplant centers, the initial dose of glucocorticoids is usually administered during surgery as intravenous methylprednisolone, at doses between 2 and 10 mg/kg body weight. The oral dose of glucocorticoids used for maintenance therapy varies between 15 and 60 mg/m^2 per day (0.5–2 mg/kg body weight per day), which is gradually tapered over time to approximately 3–5 mg prednisone per m^2 body surface area, usually taken as a single morning dose. Alternate-day dosing is often administered 6–12 months posttransplant to minimize the effect of glucocorticoids on growth.

Glucocorticoids have multiple side effects in children, including growth impairment, susceptibility to infections, Cushingoid appearance, body disfigurement, acne, cardiovascular complications, arterial hypertension, hyperglycemia, aseptic bone necrosis, osteopenia, cataracts, poor wound healing, and psychological effects (Table 3). These negative impacts on appearance may play a role in poor adherence, especially in the body image–conscious adolescents. The risk for infection is excessive if high-dose pulse therapy is prolonged (typically >3 g/1.73 m^2). Glucocorticoid dosage should, therefore, be decreased gradually during rejection treatment, even if serum creatinine fails to improve. Interestingly, glucocorticoids are not associated with increased risk for malignancy. One of the most important reasons for stopping corticosteroids or switching to alternate-day therapy is growth impairment, which is frequently observed in those on continuous treatment.

Due to the multiple adverse effects of maintenance glucocorticoid (steroid) therapy, attempts have been made to withdraw or minimize them in children with a renal allograft [90–96]. There are two major approaches in steroid minimization in pediatric renal transplantation: (i) late steroid withdrawal (>1-year posttransplantation) and (ii) either complete steroid avoidance or early steroid withdrawal (<7 days posttransplantation). In the late steroid withdrawal approach, the patients suitable for minimization are identified by stable posttransplant clinical course and renal allograft function. In late steroid withdrawal, there is no need for an antibody induction in the perioperative period [94, 95]. In early withdrawal or complete avoidance protocols, the criteria of suitability are predefined before transplantation (e.g., criteria of low immunological risk) and antibody induction is used in all enrolled patients [22, 96–100]. There is also an “intermediate” approach, combining elements from early and late withdrawal protocols, in which antibody induction is used; however, the decision of corticosteroid withdrawal is delayed until 6–9 months posttransplant, when stable renal graft function (sometimes combined with normal picture of protocol biopsy) allows to identify suitable candidates (as in the late withdrawal approach) [84, 101]. Although some hypothesize that corticosteroid withdrawal has the advantage over corticosteroid avoidance and that immunologically high-risk patients and those with unstable renal allograft function can easily be identified beforehand and be excluded from corticosteroid-free immunosuppression, others support that corticosteroid avoidance avoids the conditioning of the immune response to chronic corticosteroid exposure and may more likely to be successful and have lower immune risk than corticosteroid withdrawal, where an immune system, primed to chronic corticosteroids, now "rebounds" and develops acute rejection during the withdrawal period [102].

Corticosteroid avoidance may eventually be found to provide the best overall risk-to-benefit ratio with maintenance immunosuppressive therapy in renal transplantation. Corticosteroid avoidance protocols have been used successfully and have undergone extensive evaluation both in the USA and in Europe. However, many of these protocols have chosen low-risk individuals and utilized intensive induction therapy with thymoglobulin, tacrolimus, and MMF. Regarding the efficacy and safety of early corticosteroid withdrawal or complete corticosteroid avoidance, a randomized controlled study in 196 pediatric renal transplant recipients, two doses of daclizumab in patients treated with a regimen of tacrolimus and MMF allowed early steroid withdrawal on day five posttransplant [98]. There was a comparable rate of biopsy-proven acute rejection rates after 6 months in patients of corticosteroids compared with controls (10.2% versus 7.1%). In addition, pre-pubertal patients with early corticosteroid withdrawal showed better growth and lipid and glucose metabolism profiles compared with controls, without increases in renal allograft rejection or loss. These favorable effects were confirmed in a follow-up study over a 2-year observation period [99]. The results of the North American randomized controlled multicenter study with a follow-up of 3 years posttransplant showed that the corticosteroid-free group showed lower systolic blood pressure and lower cholesterol levels [100]. The authors concluded that complete corticosteroid avoidance is safe and effective in nonsensitized children receiving primary renal transplants [100]. Many centers now opt to utilize complete corticosteroid avoidance in the unsensitized, low-risk pediatric recipients, with 3–5 days of thymoglobulin with the recent corticosteroid-free protocol that only utilizes corticosteroids for the thymoglobulin premedication [103]. The corticosteroid avoidance regimen also provides some benefit for reducing subclinical and clinical herpes viremia, which has a positive impact for both reducing recipient morbidity from infection and improving allograft function and reducing anti-donor HLA responses [104].

Nevertheless, corticosteroid withdrawal or avoidance following renal transplantation remains a center-specific choice based on local experience with either regimen. Although the benefits of using corticosteroid-free protocols in pediatric patients shows great promise, further study is needed to determine the impact to identify new monitoring tools to assess the immunologic safety for patients who can be successfully converted to later posttransplant corticosteroid-free immunosuppression after having initially started a corticosteroid-based maintenance immunosuppression protocol, without increasing the risk of acute rejection.

Generic Immunosuppressive Drugs

In the field of solid organ transplantation, the number of immunosuppressive drugs prescribed to prevent rejection is relatively small. Not more than 10 different compounds have been used over a period of 50 years. For most of these drugs the patents have expired (azathioprine, ciclosporin, tacrolimus, mycophenolate mofetil, and sirolimus), or will expire within the next few years (everolimus and mycophenolate sodium) [105]. Policymakers consider generic drugs an attractive option to enable savings on medication cost, allowing the savings to be used for funding high-cost medicines. Generic immunosuppressive drugs are available in Europe, Canada, and the USA. Between countries, there are large differences in the market penetration of generic drugs in general, and for immunosuppressive drugs in particular. To allow for safe substitution, a number of criteria need to be fulfilled. Generic substitution should not be taken out of the hands of the treating physicians. Generic substitution can only be done safely if initiated by the prescriber, and in well-informed and prepared patients. Payers should refrain from forcing pharmacists to dispense generic drugs in patients on maintenance treatment with innovator drug. Instead, together with transplant societies, they should design guidelines on how to implement generic immunosuppressive drugs into clinical practice. Substitutions must be followed by control visits to check if the patient is taking the medication correctly and if drug exposure remains stable. Inadvertent, uncontrolled substitutions from one generic to another, initiated outside the scope of the prescriber, must be avoided as they are unsafe. Repetitive subsequent generic substitutions result in minimal additional cost savings and have an inherent risk of medication errors [105].

Adherence to Immunosuppressive Medication

Adherence to medications is defined as the process by which patients take their medication as prescribed. Adherence is of critical importance and it is often lacking, especially in teenagers. Adolescent age at transplant is an independently significant risk factor for worse long-term renal allograft survival in all major pediatric solid organ transplant types [106]. Adolescents have also been identified to have a multitude of psychiatric and socioeconomic risk factors that are deserving of greater attention and customized care delivery programs [107]. Immunosuppression obviously only works if it is taken reliably, and there is ample evidence in the literature that this reliability is lacking far too often and that such lack is leading to adverse outcomes. Moreover, immunosuppressive regimens may need to be adjusted to support adherence, for example, by combination products to reduce the pill burden and/or by once daily dosing. For example, there is a prolonged-release tacrolimus formulation available for older children and adolescents (Advagraf™ in Europe and Astagraf™ in the USA). Comparative pharmacokinetic studies have shown that stable pediatric transplant recipients can be converted from immediate-release to prolonged-release-tacrolimus at the same total daily dose, using the same therapeutic drug monitoring method [108, 109].

Diagnosis and Management of Allograft Rejection

The renal allograft, during its lifetime, is at continuous risk for the development of rejection due to HLA and non-HLA incompatibility with the recipient. Allograft rejection has been historically classified as hyperacute, acute, and chronic.

Hyperacute Rejection

Hyperacute rejection (HAR) can occur due to preformed donor-specific antibodies against HLA antigens, antibodies to ABO blood group antigens, or rarely to other minor HLA and tissue antigens [110]. Immediately upon grafting, these antibodies bind to the vascular endothelium causing complement-mediated endothelial injury, resulting in vascular clotting and renal allograft loss. Histologically, there is glomerular thrombosis, fibrinoid necrosis, polymorphonuclear leukocyte infiltration, and intra-graft hemorrhage. HAR existed in the early era of transplantation, when the HLA matching and cross-match techniques were less well developed. It has been nearly eliminated with flow-based sensitive cross- match assays and precise pre-transplant characterization of anti HLA antibodies, avoiding transplants with antigens against which these antibodies are directed.

Acute Rejection

Acute rejection denotes acute allograft dysfunction due to immune-mediated injury and generally occurs within 1 week and 6 months of transplantation, although it can occur at any time in the face of lower-maintenance immunosuppression. Advances in immunosuppressive therapy have drastically improved acute allograft rejection rates to less than 15% in the first year of transplant

[111]. Depending upon the timing of acute rejection in relation to transplant, it may be arbitrarily classified as early acute rejection (EAR) which can happen within first-year posttransplant and late acute rejection that can happen any time after that. Rates of acute rejection remain high in adolescents and young adults attributed mainly to nonadherence with the maintenance immunosuppressive therapies [112]. Acute rejection episodes may also occur when there is aggressive reduction of immunosuppression in response to viral replications such as EBV/BKV or due to calcineurin inhibitor (CNI) minimization strategies. In the modern era, there is decreased prevalence of early acute rejections due to potent induction and maintenance immunosuppression, whereas late acute rejection rates are increasing [113].

Risk factors: An individual's immunologic risk for acute rejection is attributed to overall level of HLA mismatch with the donor and presence of donor-specific anti-HLA antibodies. Repeat transplants, black race, recipient age, and delayed graft function are additional risk factors. HLA mismatch with the donor and presence of donor-specific antibodies (DSA) are the main predictors of acute T-cell-mediated cellular rejection (ATCR) and acute antibody-mediated rejection (ABMR), respectively. HLA epitope matching, especially for DR and DQ loci, is being investigated to improve immunological matching between donor and recipients and to decrease long-term risk of de novo DSA formation, ABMR, and improve renal allograft outcomes [114, 115]. Approximately 30–50% of patients with pre-transplant DSA undergoing desensitization may experience ABMR [116]. Delayed graft function has been shown to increase the risk for early acute rejection in several adult studies [117]. The single most important risk factor for late acute rejection in primary transplant recipients is nonadherence with immunosuppression, especially if combined with a high degree of HLA or epitope mismatch [118]. High coefficient of variation, expressed in percentage (%) (defined as SD /mean ×100), of tacrolimus trough levels has been correlated with an increased incidence of acute rejection and used as a marker of nonadherence [119, 120].

Clinical features: Acute rejection should be suspected when there is acute renal allograft dysfunction, manifested as an increase in serum creatinine. This increase is arbitrary and an increase of serum creatinine by 0.3 mg/dL or >15–25% increase over baseline is considered significant. The increase in serum creatinine may not occur in young children until into the late stage of acute rejection due to high nephron mass in a child with an adult-sized kidney. Other features of acute rejection may include allograft pain and tenderness, oliguria, hematuria, hypertension, and proteinuria. However, the majority of children with acute rejection are asymptomatic.

Differential diagnosis: Conceptually, acute allograft dysfunction is akin to acute kidney injury (AKI) occurring in a transplant kidney. Differences from AKI in native kidneys include unique characteristics of transplant. The transplanted kidney is a single kidney that has gone through implantation process, and is HLA incompatible with the recipient who is receiving chronic immunosuppression with nephrotoxic calcineurin inhibitor (CNI) medications. Therefore, differential diagnosis of acute allograft dysfunction includes pre-renal and post-renal causes, AR, CNI toxicity, and infections (Table 4). Sometimes, drugs that interfere with creatinine excretion such as sulphamethoxazole-trimethoprim and ciprofloxacin may also cause an increase in serum creatinine and should be considered in differential diagnosis.

Diagnosis: Laboratory studies may show an increase in serum creatinine, proteinuria, hematuria, and/or leukocyturia. Radiographic studies are nonspecific for the diagnosis but would help exclude other causes of allograft dysfunction such as obstruction to urine flow or urinary leak. Doppler renal ultrasound will help evaluate perfusion of the transplant kidney and would rule out any renal arterial or venous thrombosis (rare outside immediate posttransplant period). Otherwise, ultrasound findings of acute rejection are nonspecific and may include increased allograft size, loss of cortico-medullary differentiation, and elevated resistive indices on Doppler. Nuclear medicine scans including MAG-3 renography may demonstrate rapid uptake of the tracer but delayed

Table 4 Differential diagnosis of acute allograft dysfunction

Pre-renal
Dehydration, low BP Renal arterial/venous thrombosis (rare outside immediate post-op period)
Renal
Acute rejection, CNI toxicity Infections: APN, BKV, CMV and adenovirus[a] Recurrent diseases: FSGS, aHUS, C3GN, primary hyperoxaluria, IGAN[a], ANCA+ GN[a], anti GBM disease[a], De novo diseases: FSGS, Membranous GN
Post-renal:
UPJ or UVJ obstruction, perirenal hematoma or fluid, stent obstruction or dislodgement Bladder dysfunction
Risk factors for chronic allograft dysfunction
Immune factors: Chronic active TCMR (HLA A, B, DR mismatch or high epitope mismatch load) Chronis active ABMR (donor specific antibodies, antibodies to ATII type I receptor, anti- endothelial cell antibodies, MICA antibodies and rarely other autoantibodies)
Non-Immune factors:
Donor factors (age, race, size, cause of death) Transplant related factors (ischemia reperfusion injury) Chronic CNI toxicity, chronic viral infection (BKV), Recurrent pyelonephritis Recurrent obstructions (UPJ or UVJ obstruction or bladder dysfunction) Recipient factors (High BMI, hypertension, proteinuria, diabetes, dyslipidemia and rarely chronic hypoperfusion in young children due to low blood pressure) Recurrent diseases (FSGS, aHUS, C3GN, hyperoxaluria)

Abbreviations: *aHUS* atypical hemolytic uremic syndrome, *ABMR* antibody mediated rejection, *ANCA* antineutrophilic cytoplasmic autoantibody, *APN* acute pyelonephritis, *BKV* BK virus infection, *CMV* cytomegalovirus, *C3GN* C3 glomerulonephritis, *CNI* calcineurin inhibitors, *EBV* Epstein Barr virus, *FSGS* focal segmental glomerulosclerosis, *GBM* glomerular basement membrane, *GN* glomerulonephritis, *IGAN* IgA nephropathy, *MICA* minor histocompatibility chain A, *TCMR* T-cell-mediated rejection, *UPJ* ureteropelvic junction, *UVJ* ureterovesical junction

[a] Rare

cortical function and no obstruction to urine flow. Radionuclide scans and ultrasounds cannot distinguish among various causes of graft dysfunction, such as rejection, acute tubular necrosis, CNI toxicity, and viral nephropathies. Other tests such as CNI levels, COV% of tacrolimus trough levels, urinalysis and culture, and blood BK DNA viral load may give clues to the differential diagnosis.

Renal allograft biopsy remains the gold standard diagnostic procedure. In addition to confirming the presence or absence of acute rejection, histopathology allows evaluation for the two main forms of acute rejection: ACR and ABMR. As the mechanisms and therapies for ACR and ABMR differ, the importance of defining the underlying pathology cannot be understated. Of note, distinguishing between severe ACR and ABMR can be challenging, and both may coexist especially in LAR. Allograft histology is interpreted using the Banff classification which has undergone extensive updating and revisions [121, 122].

Acute Cellular Rejection

Histopathology of ACR is characterized by interstitial infiltration with mononuclear cells and tubulitis (disruption of the tubular basement membrane by infiltrating cells), and intimal arteritis (subendothelial infiltration of mononuclear cells). Occasional eosinophils and plasma cells may be present, though predominance of eosinophils may be suggestive of interstitial nephritis. Areas of patchy interstitial inflammation without evidence of tubulitis are not uncommon and are insufficient to support diagnosis of ACR. The presence of significant number of neutrophils may suggest pyelonephritis or ABMR. Banff classification of ACR defines this into borderline, grade I A and 1B, depending upon the area of interstitial inflammation and severity of tubulitis

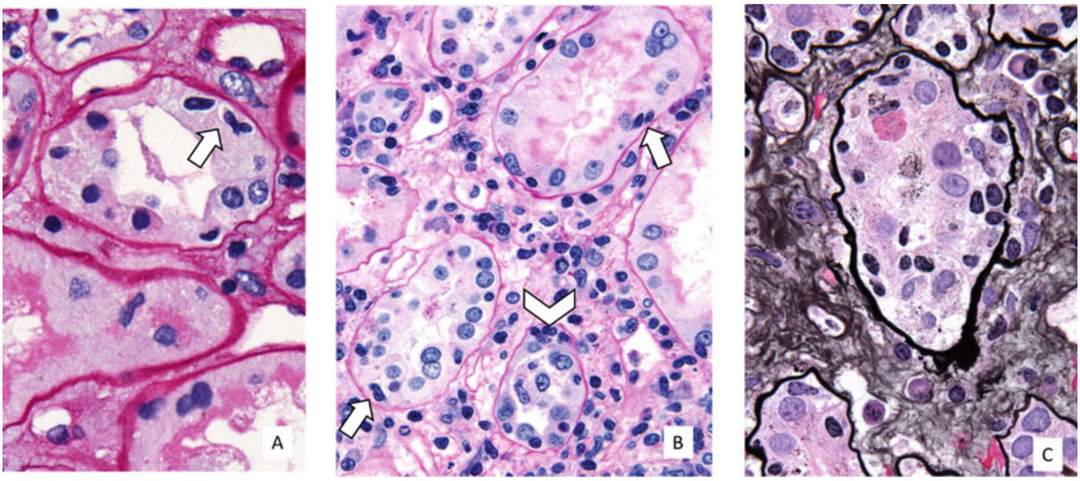

Fig. 3 Acute T-cell-mediated rejection. (**a**) Mild tubulitis with 1–4 lymphocytes (arrow) in a tubular wall. (PAS) (**b**) Interstitial inflammation with mild (arrows) and moderate (5–10 lymphocytes/10 tubular cells, chevron) tubulitis, Banff grade 1A. (PAS) (**c**) Severe tubulitis with >10 lymphocytes/10 tubular cells, Banff grade 1B. (Jones silver stain). (Courtesy, Dr. Cynthia Nast, Cedars-Sinai Medical Center, LA, CA)

(Fig. 3) and into grade 2A, 2B, and 3 depending upon the involvement and severity of arteritis (Table 5).

Acute Antibody-Mediated Rejection

ABMR may occur due to formation of de novo DSA after transplant or due to preexisting DSA in patients undergoing desensitization and rarely due to other antibodies. These antibodies interact with endothelial cells of glomerular and peritubular capillaries (PTC) and may cause complement-mediated or antibody-dependent cytotoxic damage depending upon the strength and characteristics of antibodies. The Banff classification of acute ABMR continues to evolve with recognition of its variable histologic presentation. The diagnosis of acute ABMR depends upon the presence of all three criteria (histologic evidence of injury, current/recent interaction of DSA with the endothelium, and serologic presence of DSA) shown in Table 5. Histologic changes include presence of either acute tubular injury or glomerulitis and peritubular capillaritis or intimal arteritis or thrombotic microangiopathy without an apparent cause. Current/recent interaction of DSA with the endothelium includes presence of C4d (complement 4 split product) deposited in PTC demonstrated by immunofluorescence or immunoperoxidase or presence of moderate microvascular inflammation or gene transcripts of endothelial activation (Fig. 4). Though C4d-positive ABMR may represent a more severe form, the sensitivity of C4d is low due to its focal presence and dependency on the PTC for expression and ability of DSA to bind complement. Studies have established the existence of C4d-negative ABMR. Therefore, C4d staining is no longer a requirement for the diagnosis of ABMR and has been substituted with either presence of endothelial cell transcripts (not widely available) or presence of moderate microvascular inflammation [121].

Noninvasive Biomarkers of Acute Rejection

Noninvasive monitoring of blood and urine for prediction of acute rejection and graft injury is being investigated by using gene expression panels, immune repertoire sequencing [123, 124], transcriptomes [125], proteomes [126], and donor-derived cell-free DNA in blood [127–129]. In addition, a recent publication this year identified a composite panel of cell-free DNA, methylated cell-free DNA, CXCL10, clusterin, protein, and creatinine in urine [127], to result in a composite risk score (Q-Score) to classify biopsy-confirmed ACR and ABMR in both adult and children with high levels of accuracy [130–132]. These

Table 5 Histologic diagnosis of acute allograft rejection according to Banff 2017

Acute T cell–mediated rejection (TCMR)
Ia >25% Interstitial inflammation with moderate tubulitis (t2)
Ib >25% Interstitial inflammation with severe tubulitis (t3)
IIa Mild-to-moderate intimal arteritis (v1)
IIb Severe intimal arteritis (v2)
III Transmural arteritis and/or fibrinoid necrosis (v3)
Acute antibody-mediated rejection (ABMR): Should have all three criteria below
Histologic evidence of tissue injury:
Acute tubular injury
Microvascular inflammation ($g > 0$ and/or $ptc > 0$)
Arteritis ($v > 0$)
Thrombotic microangiopathy without apparent cause
Evidence of current/recent antibody interaction with endothelium:
Positive C4d staining of peritubular capillaries
Moderate microvascular inflammation ($g + ptc \geq 2$)
Increased expression of gene transcripts in biopsy tissue strongly associated with ABMR
Serologic evidence of donor-specific antibodies (DSA)
Positive C4d staining/ presence of ABMR-associated gene transcripts may substitute for DSA

i0 – No inflammation or in <10% of unscarred cortical parenchyma. i1 – Inflammation in 10–25% of unscarred cortical parenchyma. i2 – Inflammation in 26–50% of unscarred cortical parenchyma. i3 – Inflammation in more than 50% of unscarred cortical parenchyma

t0 – No mononuclear cells in tubules or single focus of tubulitis only, t1 – Foci with 1–4 mononuclear cells/tubular cross section (or 10 tubular cells), t2 – 5–10 mononuclear cells and t3 – Foci with >10 mononuclear cells/tubular cross section or the presence of ≥2 areas of tubular basement membrane destruction accompanied by i2/i3 inflammation and t2 elsewhere

G: glomerulitis, none (g0), segmental or global glomerulitis in 75% of glomeruli (g3), PTC: peritubular capillaries- at least 1 leukocyte in ≥10% of cortical PTC but 10 leukocytes in most severely involved PTC (ptc3)

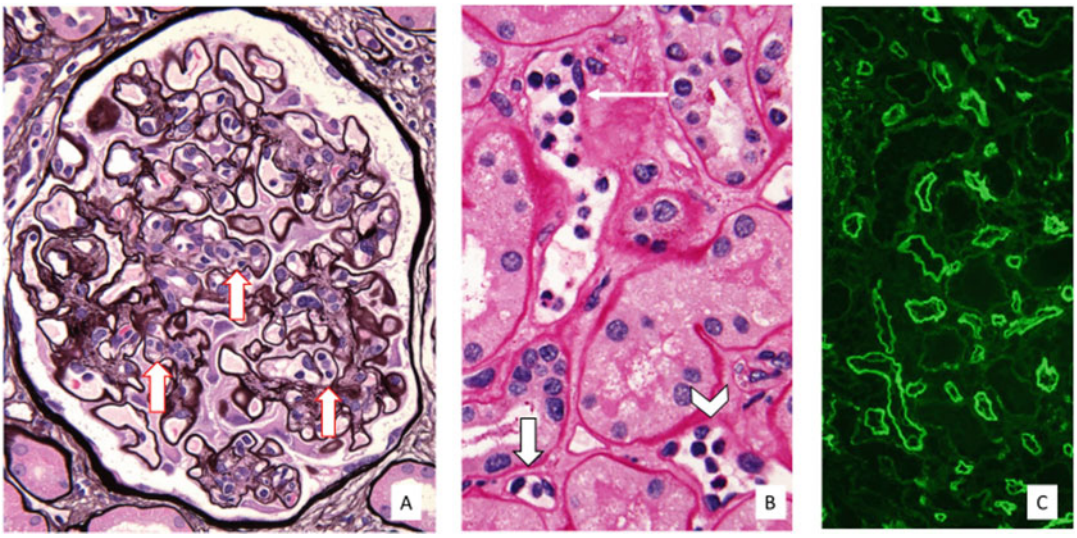

Fig. 4 Active antibody mediated rejection. (**a**) Leukocytes, predominantly monocytes, and swollen endothelial cells(arrows) are within and occluding glomerular capillary lumens (glomerulitis). (Jones silver) (**b**) Leukocytes and swollen endothelial cells in peritubular capillary lumens (peritubular capillaritiis). Mild (3–4 cells, thick arrow); moderate (5–10 cell, chevron); severe (>10 cells, thin arrow). (PAS) (**c**) Immunofluorescence for C4d showing linear staining of peritubular capillary walls. (Courtesy, Dr. Cynthia Nast, Cedars-Sinai Medical Center, LA, CA)

biomarkers are being currently studied in children to pave the way over time to early and noninvasive diagnosis of acute rejection in the near future [133].

Therapy

Treatment strategies depend upon whether ACR, acute ABMR, or mixed acute rejection is present. Aggressiveness of treatment generally follows severity of lesions. Graft prognosis after treated acute rejection also depends on type and severity; however, any untreated clinical acute rejection episode will ultimately result in accelerated graft loss. Thus, timely diagnosis is crucial to promptly initiate appropriate treatment.

Standard initial treatment of ACR is intravenous methylprednisolone (10 mg/kg body weight, maximum dose, 0.5–1.0 g) as a single daily dose for 3–5 days. Majority of Banff class 1 lesions respond to methylprednisolone alone. This is typically followed by the intensification of oral prednisone (i.e., 2 mg/kg/daily) and subsequent taper to pre-rejection dosage [134]. For children who are on steroid-free immunosuppression prior to acute rejection, it may be reasonable to stop prednisone. However, if episodes of rejection prove to be recurrent or DSA is detected, continuation of low-dose-maintenance steroids may be needed. If renal function does not improve after 5 days of intravenous glucocorticoids, steroid-resistant rejection should be considered.

Steroid-resistant acute ACR and Banff II and III lesions generally require T cell–depleting therapy, most commonly rabbit antithymocyte globulin (rATG) dosed at 1.5 mg/kg for five to seven doses. A randomized, controlled trial showed superior efficacy of rATG over horse antithymocyte globulin [135]. Cochrane Database review in 2017 concluded that antibody therapy was superior to steroid therapy in reversing T cell–mediated rejection with no effect on subsequent acute rejections [136]. There are scant pediatric data to guide dosing. Data using alemtuzumab for treatment of acute rejection are limited but suggest efficacy [137]. Lymphocyte depleting antibodies often produce cytokine release effects including fever and chills. Methylprednisolone, antihistamines, and acetaminophen are used to decrease these side effects. Due to potent immunosuppressive effect of rATG, there is concern for increased risk of infection and lymphoproliferative disorder [138]. Posttreatment viral monitoring and prophylaxis against cytomegalovirus and pneumocystis jirovecii should be provided until lymphocytes recover to the reference range, usually for 4–6 months.

The treatment of acute ABMR is much less efficacious and aims of treatment are threefold: (1) to remove existing alloantibodies, (2) to prevent damage being caused by existing alloantibodies, and (3) to prevent ongoing production of alloantibodies. Therapeutic approaches include combinations of plasmapheresis, immunoadsorption, intravenous immunoglobulin G (IVIG), anti-lymphocyte therapies, and adjustment of baseline immunosuppression. Many other experimental therapies have been tried in small studies.

Treatments of ABMR trials are small with large degrees of heterogeneity including patients with both acute and chronic lesions [139]. There is low-quality evidence supporting plasma exchange, and very-low-quality evidence for all other treatments. Nevertheless, plasma exchange and IVIG, with or without B cell depletion with intravenous rituximab, is the most used strategy for ABMR. A typical regimen includes daily or every other day plasma exchange consisting of 1.5 plasma volume removals with each treatment followed by IVIG at 100–200 mg/kg, with or without a single dose of rituximab at 375 mg/m^2. Immunoadsorption is no longer available in the USA.

IVIG is shown to be beneficial especially when used in combination with other therapies though there are no randomized controlled studies to support its use. Proposed mechanisms of IVIG include the suppression of immunoglobulin synthesis, altered complement binding, and DSA neutralization. Plasmapheresis removes antibodies from circulation, though may stimulate rebound antibody production and should not be used as monotherapy and may offer benefit when used in combination with other therapies. Some studies suggest addition of rituximab [140], whereas others have used bortezomib, eculizumab, or anti-IL-6 antibody added to multimodal therapy [141]. Complement inhibitor therapy has been tried to rescue severe ABMR

[142]. Presently, there is insufficient evidence to support consensus guidelines for the treatment of ABMR. Experts recommend that for patients with allograft dysfunction and risks for ABMR (positive DSA, history of desensitization therapy, and previous transplant) who have biopsy evidence of antibody-mediated injury (C4d+) but minimal pathologic findings (ATN-like changes), treatment should be initiated with high-dose IVIG (2 g/kg) and one dose of rituximab (375 mg/m^2). For those patients with more severe clinical features and pathology, plasma exchange is provided in addition to IVIG and rituximab [139]. Other experimental therapies may be considered but risks versus benefits should be evaluated prior to such considerations [140].

Chronic Rejection or Chronic Allograft Dysfunction

Chronic rejection is included in the broader term chronic allograft dysfunction (CAD) due to recognition that it may not be feasible to differentiate between allograft injury caused by immune and nonimmune causes. Therefore, CAD is a generic term that includes all causes of chronic renal allograft dysfunction associated with fibrosis [143, 144], all causes shown in Table 4. CAD is the most common cause of graft loss in children [143].

The clinical diagnosis is generally suspected when there is gradual deterioration of allograft function (“creeping creatinine”), increasing proteinuria, and worsening hypertension. This is akin to chronic CKD in native kidneys except for presence of additional alloimmune-mediated injury (chronic active T-cell- and antibody-mediated injury). Clinical findings can be insensitive and often underestimate the severity of the underlying pathologic change, and late diagnosis accompanied by progressive pathologic change is expected to lead to graft loss despite therapeutic efforts to intervene.

The pathologic findings of CAD involve all components of renal parenchyma: vasculature, glomeruli, tubules, and interstitium [144]. The glomeruli may be enlarged with a lobular appearance and may include thickened capillary walls with double-contour appearance. Electron microscopy may reveal mesangial cell interposition and endothelial cells swelling and increased thickening and multilayering of glomerular basement membrane. These findings resemble those of membranoproliferative glomerulonephritis, though there are no immune deposits. Immunofluorescence staining may show C4d, which suggests ongoing chronic antibody-mediated injury. Peritubular capillary basement membrane shows splitting and lamination again due to repeated injury and repair. Varying degrees of interstitial fibrosis and tubular atrophy is noted, and focal cellular infiltrate with lymphocytes and plasma cells are often present. Vascular changes include subintimal accumulation of connective tissue and proliferation of myofibroblasts, resulting in thickening of the vessel walls; the presence of vasculopathy has been associated with worse graft outcomes.

The pathogenesis of CAD is complex and involves both immune and nonimmune factors as contributors. Chronic graft injury due to presence of DSA is a major contributor to CAD, and the role of minor HLA antigens and non-HLA antigens in the pathogenesis is less well studied [145, 146]. Chronic CNI toxicity and BKV nephropathy are important contributors to CAD.

Given the limited impact of treatment of CAD, prevention remains an important goal. Individual recipient risks should be considered when planning immunosuppressive regimens with the aim of preventing acute rejections while minimizing CNI nephrotoxicity and BKV nephropathy. Prevention of acute rejection by promoting adherence is an extremely important intervention and requires multidisciplinary approach. Regimens minimizing exposure to CNI from the time of transplantation have not produced favorable results [147], though may be more efficacious with contemporary agents such as belatacept. Converting from CNI to mTOR inhibitors posttransplant appears most effective in patients with relatively preserved renal function (GFR >40 mls/min/1.73 m^2) and limited or absent proteinuria [148]. Furthermore, the use of mycophenolate over azathioprine has been shown to be beneficial in reducing the incidence of CAD at 1 year [149]. With respect to prevention and interventions of nonallogenic risk factors, appropriate donor selection for pediatric recipients should minimize risks associated with poor donor quality. Pediatric preference in allocation favors the allocation of kidneys from high-quality deceased donors to children. With respect to recipient factors, blood pressure should be monitored and treated [150]. Treatment of proteinuria in pediatric renal transplant patients with ACEI or ARB should be considered. These recommendations are based upon studies demonstrating delay of progression of CKD in children and are not specific to pediatric transplant patients [151]. Additional measures include tight control of weight, lipids, and glucose [152]. In conclusion, prevention of both immune- and nonimmune-mediated causes of CAD should be the prime focus to help maintain longevity of the graft since treatment is less than optimal.

Conclusions and Outlook

Transplantation in children carries unique challenges. While issues such as controlling rejection and minimizing side effects are similar between adults and children, maintenance immunosuppressant regimens that affect developmental processes have a disproportionate impact on children. This is particularly true for glucocorticoids, which have many side effects including some that can be quite devastating in pediatric patients. Corticosteroid avoidance has been very successful, when MMF is combined with tacrolimus and either basiliximab or antithymocyte globulin for induction therapy is added; long-term follow-up on these patients with regards to improvements in renal allograft survival and graft function will be very informative, as corticosteroid-free patients also have reduction of comorbidities that drive CAN. With the goal of eliminating steroids, the combination of MMF and tacrolimus may strike the correct balance between adequate and over-immunosuppression. Recently, the use of everolimus has been advocated for minimizing calcineurin inhibitor exposure after renal transplantation, but its use is limited by mTOR-related side effects. There is at present no consensus for immunosuppressive therapy following renal transplantation in children.

Newer drugs such as belatacept have not been systematically studied in the pediatric transplant patient population. Since the approval of belatacept in 2011 for use in de novo adult renal transplantation, this CD80/86–CD28 co-stimulation blocker has been shown to be a valuable treatment option for maintenance immunosuppression [153]. Belatacept in adults has been associated with superior glomerular filtration rate as compared to calcineurin inhibitor–based treatments because of the absence of nephrotoxicity. Additionally, belatacept avoids the cardiovascular side effects (e.g., hypertension and dyslipidemia) caused by a calcineurin inhibitor–based regimen [153]. However, its use is limited to EBV-seropositive patients, since EBV-naive patients on belatacept have an increased risk for PTLD. Nevertheless, there is great interest to bring in belatacept as possible "depot" immunosuppression in the adolescent pediatric recipient, a population that have the highest rates of renal allograft loss and will more likely be plagued with immunosuppression nonadherence and had prior exposure to EBV. Belatacept is safe in adolescents but not yet approved for children or EBV-naive patients given additional risk for developing PTLD [154]. Studies are underway to explore safe protocols for the use of belatacept in adolescent children.

Clearly, much additional work is needed to define optimal immunosuppressive regimens in pediatric renal transplant patients, particularly with respect to newer and evolving regimens. The safety and efficacy of these protocols with special emphasis on long-term renal allograft survival, PTLD, and other malignancies need to be established.

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Recurrent Disease After Pediatric Kidney Transplantation

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Debora Matossian, Patrick D. Brophy, and Priya Verghese

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Abstract

Pediatric kidney transplantation is a lifesaving therapy for children with kidney failure. With modern immunosuppression, transplant rejection is as low as 11% in the first year post-transplant. Data from the United States Scientific Registry of Transplant Recipients (SRTR) reported improvements of one-year graft survival for transplants performed in 1987 versus 2010 from 81% to 97%, and 10-year graft survival for transplants performed in 1987 versus 2001 (47% versus 60%) (Pediatrics 133(4): 594–601, 2004). Transplant offers numerous quantifiable and non-quantifiable benefits over dialysis. But some diseases that cause end stage kidney diseases can recur after transplant. The effects of this disease recurrence on patient and kidney allograft health are often devastating. Recurrence of disease accounts for 7–8% of graft losses in pediatric kidney transplant recipients and is the fourth most common cause of graft loss after chronic rejection, acute rejection, and death with a functioning graft (Pediatr Nephrol 24:2097–2108,

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2009). This chapter is focused on describing the incidence and pathogenesis of some of these diseases in greater detail and providing an updated literature review on potential preventive and therapeutic strategies for recurrent disease in the kidney allograft.

Keywords

Recurrence · Kidney · Transplant · Pediatric · Graft

Diseases that Affect the Glomerular Microvasculature

Atypical Hemolytic Uremic Syndrome (aHUS)

Hemolytic Uremic Syndrome (HUS) in children is a disease characterized by endothelial swelling. This microvascular inflammation initiates a cascade of platelet aggregation with consequent consumptive circulating thrombocytopenia, turbulent non-lamellar blood flow resulting in hemolytic anemia and renal failure from microvascular occlusion. The inciting event which causes the endothelial injury varies. In children, it is most commonly caused by an enteropathic shiga-toxin producing bacteria such as *E. coli* 0157:H7 (STEC), *shigella dysenteriae*, or *campylobacter jejunii* or rarely organisms such as Streptococcus pneumoniae. This type of HUS is also called typical HUS because is it more common or D+ HUS, referring to the usual occurrence of diarrhea as a preceding event [1, 2]. Progression to end stage kidney disease (ESKD) is less common in this type of HUS and it almost never recurs in the kidney transplant (<1%) [3]. On the contrary, non-shiga-toxin associated HUS has a higher incidence of progression to ESKD (50%) and recurrence after kidney transplantation (80%) [4] and will be the focus of this section.

Atypical HUS (aHUS) is an ultra-rare disease with a reported incidence of approximately 0.5 per million per year. It is caused by dysfunction in the alternate complement pathway which causes uncontrolled complement activity resulting in endothelial injury and swelling with the consequent triggering of the cascade described above. The alternate complement pathway dysfunction is due to mutations in factors of the complement system or antibodies directed against complement factors in 60–70% of patients [5]. On biopsy, classic features of thrombotic microangiopathy (TMA) are observed. Once biochemical, hematological and pathology evaluations have demonstrated TMA, the focus should be on determining the underlying etiology and excluding other diagnoses. The most urgent requirement is to measure ADAMTS13 activity to diagnose or exclude thrombotic thrombocytopenic purpura. Also, infectious evaluation for STEC-HUS should be performed since 5% have no prodromal diarrhea. Serum levels of complement proteins should be measured. Complement functional assays and activation markers can also be obtained. However, delays in obtaining results from genetic or molecular diagnostic studies should not prevent a clinical diagnosis or postpone treatment, as early anti-complement treatment is crucial to preserve renal function and avoid irreversible sequelae. Genes that should be screened include CFH, CD46, CFI, C3, CFB, THBD, CFHR1, CFHR5, and DGKE. The identification of a pathogenic genetic variant in a patient with aHUS reinforces the diagnosis and establishes with accuracy the cause of disease, facilitating patient management, effective treatment, and genetic counseling.

Once ESKD is confirmed, kidney transplantation can be pursued. But caution is merited, and transplantation should be delayed at least 6 months after the start of dialysis, since renal recovery may be observed at varying times after the starting anti-complement therapy. The resolution of hematological TMA features and extra-renal manifestations is a prerequisite for transplantation and special consideration required before pursuing a living donor. Living-related kidney donation carries a risk in the recipient and a risk of de novo disease in the donor, should the donor carry an at-risk genetic variant. Potential donors with evidence of abnormal alternative complement pathway should be excluded.

The risk of aHUS recurrence after transplant varies depending on the mutation identified

Table 1 Risk of aHUS recurrence according to the implicated genetic abnormality

Complement Gene Mutation	Location	Functional Impact	Mutation frequency in aHUS (%)	Recurrence after Transplantation (%)	aHUS & C3GN	Prophylactic eculizumab recommendation
CFH	Plasma	Loss	20–30	75–90	Yes	Yes
CFI	Plasma	Loss	2–12	45–80	Yes	Yes
CFB	Plasma	Gain	1–2	100	Yes	Yes
C3	Plasma	Gain	5–10	40–70	Yes	Yes
MCP	Membrane	Loss	10–15	15–20	Yes	No
THBD	Membrane	Loss	5	1 case	Only aHUS	Yes
CFHR1–5	Circulating	Undetermined	14–23	NA	Yes	Yes

Adapted from Salvadori M, Bertoni E. Complement related kidney diseases: Recurrence after transplantation. World J Transplant. 2016;6:632–645.

aHUS: atypical hemolytic uremic syndrome; C3GN: C3 glomerulopathy; NA: Not available; CFH: complement factor H; CFI: Complement factor I; CFB: complement factor B; C3: Complement component 3; MCP: Membrane cofactor protein; THBD: Thrombomodulin.

(Table 1), occurring most often in patients with gene mutations that encode circulating complement factors (C3, Complement Factor H (CFH), complement factor I) as compared to patients with gene mutations that encode solid phase proteins [6]. TMA could develop at any time in the post transplantation course. TMA manifestations are quite variable and can vary from a limited form confined to the kidney to a full-blown systemic variant. The systemic form of TMA consists of the classic triad of thrombocytopenia, microangiopathic hemolytic anemia, and acute kidney injury. Lactic acid dehydrogenase (LDH) can be elevated with or without a drop in haptoglobin with schistocytes on peripheral blood smear all markers for intravascular hemolysis. Localized TMA presents with non-specific graft dysfunction necessitating the diagnostic allograft biopsy.

Therapies and preventive measures for recurrent aHUS in the allograft include the use of plasma exchange, rituximab (used for aHUS due to anti-factor H antibodies, to help maintain low levels of antibodies), simultaneous liver-kidney transplant (for CFH mutations) [7], and eculizumab. Eculizumab has revolutionized the management of aHUS. Eculizumab is a humanized monoclonal antibody directed against complement protein C5; it inhibits the terminal complement activation by preventing the formation of C5b-9 or membrane attack complex. Prior to the use of eculizumab, patients with recurrent aHUS post-transplant with gene mutations in CFH, CFH-CFHR1–3, CFI, and CFB had 50% risk of progression to ESKD in the first year, which increased to 75% by 3–5 years [8]. Now eculizumab is not only the treatment of choice for aHUS but also for patients with recurrent aHUS. TMA event free status and complete TMA response has been observed in respectively >92 and 50–85% of the aHUS patients who receive eculizumab. Unfortunately, even with eculizumab, graft loss is prevalent in patients with mutations on CFH and CFH/CFHR1 [9]. Plasma exchange does not address the underlying cause of complement dysfunction but can be effective if aHUS is due to anti-FH antibodies, where plasma exchange and adequate immunosuppression effectively reduces the levels of circulating antibodies and inhibits further production [10]. Otherwise, it is only indicated when eculizumab is not immediately available.

Patients with no prior history of aHUS may also present with de novo aHUS after kidney transplantation with classic findings of TMA on pathology and does not represent recurrent disease. Calcineurin inhibitors (CNI) have been implicated by the following mechanisms: loss of the normal balance between the vasodilator peptides (e.g., prostaglandin E2 and prostacyclin) and the vasoconstrictor peptides (e.g., thromboxane A2 and endothelin), results in arteriolar vasoconstriction [11], renal ischemia, and establishment of endothelial injury [12]; CNI-induced platelet activation, pro-coagulant, and anti-fibrinolytic activity have been shown to be involved in TMA evolution, particularly so, with an injured endothelium due to antibody-mediated rejection, ischemia-reperfusion injury, or any other etiology [13]; and microparticle production from endothelial cells, a known effect of Cyclosporine A that can result in activation of the alternate complement pathway, a well-known mechanism that is implicated in TMA evolution [14].

Genetic testing has offered patients with aHUS the advantage of the prophylactic use of eculizumab. Currently, it is recommended in all kidney transplant patients at high-risk of recurrence based on their genetic mutations [15] which includes patients with previous early recurrence and those with previously described pathogenic mutations (Table 1). For those with moderate risk, including patients with no mutations identified, isolated CFI mutations, or insignificant complement gene mutations, the use prophylactic eculizumab is left to the discretion of the clinician. Low-risk mutations, which includes isolated MCP mutations and persistently negative FH autoantibodies, do not need prophylactic eculizumab. Patients who receive prophylactic use before/during transplant have better graft function than those who received eculizumab with aHUS recurrence [16]. Tight blood pressure control and likely lower tacrolimus levels could be recommended in patients with aHUS; since CNIs are now known to cause de Novo TMA as explained above. Duration of eculizumab has been controversial, and so far, it is based on expert opinion. Transplant patients are not good candidates for treatment cessation since discontinuation has been associated with aHUS relapse, particularly in patients with pathologic mutations (vs. those with no identified variants) [17]. Maintenance eculizumab requires infusions every 2 weeks. A new long-acting C5 inhibitor, Ravulizumab (Ultomiris™) has been engineered with increase elimination half-life allowing for extended intervals (from every 2 weeks to every 4–8 weeks infusions). Side effects associated with C5 inhibitors are increased risk of infections from encapsulated bacteria [18]. The risk of meningococcal disease in eculizumab recipients is 1,000 to 2,000 times greater compared to otherwise healthy individuals in the United States. Patients should receive meningococcal vaccination, including type B, ideally 2 weeks prior to initiation of eculizumab therapy. Since it is unknown whether anti-meningococcal antibodies are protective in the setting of complement blockade, it is recommended to remain on Penicillin while on C5 inhibitor and up to 2–3 months after discontinuation. However, vaccination should not delay the start of eculizumab. The following agents are under investigation: The anti-C3b blocker, compstatin analog Cp40, and anti-C3 convertase monoclonal antibodies.

Glomerular Diseases with Potential to Recur

FSGS

Focal segmental glomerulosclerosis (FSGS) is the most common cause of steroid resistant nephrotic syndrome leading to end-stage kidney disease (ESKD). Data from the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) show that FSGS carries the highest likelihood of progressing to ESKD, exceeding the risk of hypoplasia, obstructive uropathy, and reflux nephropathy. According to NAPRTCS, nearly 60% of such children with a diagnosis of FSGS progress to dialysis or transplantation within 24 months of entry into the registry [19]. Incidence of nephrotic syndrome in children is about 20 cases per million [20]. It is estimated that 20% of these cases are FSGS. In adults, the estimated incidence of FSGS is 7 per million [21]. An estimation of the incidence and prevalence of FSGS in children is hampered by the fact that most children with nephrotic syndrome, unlike the majority of adults, are not routinely subjected to renal biopsy. Therefore, prevalence and incidence figures are extrapolated from clinical reports, registries, and renal biopsy materials [22]. In addition, FSGS is a pathologic finding that can be caused by diseases that are immunologic, genetic, or other. Therefore, the reported frequency varies significantly in the literature, since many different entities culminate in a similar biopsy phenotype on biopsy [23]. FSGS can be classified as primary and secondary. Primary (idiopathic) FSGS has been attributed to an elusive circulating factor. Secondary FSGS occurs due to viruses (human immunodeficiency virus type 1, parvovirus and other less characterized such as simian virus 40, cytomegalovirus and Epstein-Barr virus), drugs (heroin, bisphosphonates, interferon therapy, mammalian target of rapamycin), and secondary to hemodynamic adaptations (due to reduction number of functioning nephron units) or hemodynamic stress (due to morbid obesity, cyanotic heart disease, sickle cell anemia). In the case of secondary FSGS, the treatment usually requires management of the underlying cause. If transplant is necessary in these patients, recurrence is rare if underlying disease/cause is treated. The familial (genetic) forms result from abnormalities in gene transcription controlling assembly of the slit diaphragm, cytoskeleton, and adhesion complexes, all of which are essential for podocyte function, in maintaining a filtration barrier. A transplant therefore is curative without risk of recurrence in genetic FSGS. This section will mainly cover the entity of primary FSGS (Fig. 1; Table 2).

Based on current data, FSGS has poorer transplant outcomes than most other causes of ESKD [24], largely because of disease recurrence in the transplanted kidney, affecting 15–36% of pediatric transplants [25–27]. The etiology of recurrence is unknown. Given the recurrence rate after kidney transplant, the presence of a circulating permeability factor has been studied [28]. There have been published reports of patients with FSGS who developed immediate recurrence of nephrotic syndrome. In some cases, the graft was removed and re-transplanted in a patient with a different cause of ESKD with excellent outcomes. This provides strong evidence of the presence of a circulating factor as the pathogenic mechanism for the recurrence of FSGS posttransplant [29, 30]. A serum soluble urokinase receptor (suPAR) has been proposed as the factor leading to foot process effacement and proteinuria. Nevertheless, the diagnostic test accuracy of suPAR remains suboptimal in predicting FSGS recurrence, [31] and this has been countered by others.

Unfortunately, we are unable to predict recurrence, but there are identifiable risk factors including an early onset of nephrotic syndrome; presentation as a child and rapid progression to ESKD. Data demonstrate that 50% of those whose progression from diagnosis to ESKD occurs in

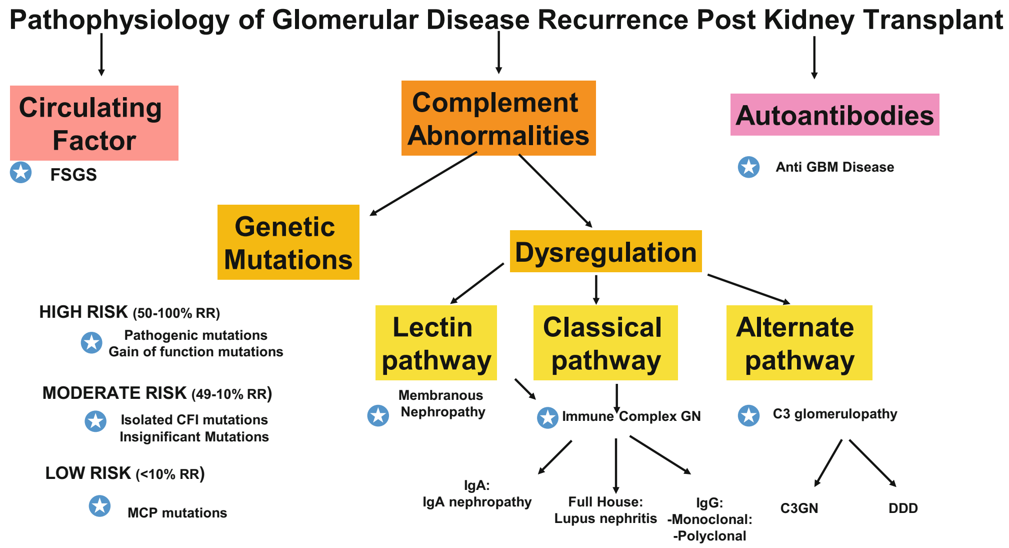
Pathophysiology of Glomerular Disease Recurrence Post Kidney Transplant
Circulating Factor
FSGS
Complement Abnormalities
Autoantibodies
Anti GBM Disease
Genetic Mutations
Dysregulation
HIGH RISK (50-100% RR)
Pathogenic mutations
Gain of function mutations
MODERATE RISK (49-10% RR)
Isolated CFI mutations
Insignificant Mutations
LOW RISK (<10% RR)
MCP mutations
Lectin pathway
Classical pathway
Alternate pathway
Membranous Nephropathy
Immune Complex GN
C3 glomerulopathy
IgA:
IgA nephropathy
Full House:
Lupus nephritis
IgG:
-Monoclonal:
-Polyclonal
C3GN
DDD

Fig. 1 Pathophysiology of Glomerular Disease Recurrence Post Kidney Transplant. FSGS: Focal Segmental Glomerulo Sclerosis; GBM: Glomerular basement disease; CFI: Complement factor I; MCP: membrane co-factor protein; GN: Glomerulonephritis; C3GN: Complement 3 glomerulonephritis; DDD: Dense deposit disease; RR: Recurrence Risk

Table 2 Summary of recurrent diseases after kidney transplantation

	Pathogenesis	Frequency %	Risk Factors	Clinical Presentation	Treatment
aHUS	Pathogenic mutations on complement pathway Antibodies against complement factors	80	Family history, pathogenic mutations	Microangiopathic hemolytic anemia Thrombocytopenia Allograft dysfunction Allograft loss	Plasma Exchange Rituximab Eculizumab Kidney +Liver Tx
FSGS	Circulating factor	15–36	Younger, rapid progression to ESKD, resistance to treatment, initial steroid sensitivity but late non-responders, non-black ethnicity and recurrence in previous transplants.	Proteinuria Hypoalbuminemia ATN Graft Loss Small vessel thrombosis	Plasmapheresis Rituximab LDL-apheresis
C3G	Dysregulation of complement alternate pathway	45	Younger age at presentation Crescents on original biopsy	Allograft dysfunction	Eculizumab?
MN	Dysregulation of complement lectin pathway	30–45	High titer of anti-PLA2R antibody, rapid progression of native ESKD and prior allograft failure secondary to recurrent membranous nephropathy	Proteinuria Allograft dysfunction Subclinical histological recurrence	Anti-proteinuric agents Corticosteroids Rituximab CNI Alkylating agents
MPGN1	Dysregulation of complement classical pathway	20–30	Younger recipients	Benign	Plasmapheresis Steroids
IGA	Dysregulation of complement classical pathway	16–60	Younger age, zero-HLA mismatched live-related donor kidneys, steroid-avoidance or early steroid-withdrawal, non-use of induction therapy, crescentic nephropathy	Benign Microscopic hematuria Subclinical histological recurrence	CNI corticosteroids
OX	Oxalate deposits	n/a	Genetics, kidney tx alone	Oxalate deposits on graft	Liver-kidney TX

aHUS Atypical hemolytic uremic syndrome, *FSGS* Focal segmental glomerulo sclerosis; *C3G* C3 glomerulopathy; *MN* Membranous nephropathy; *MPGN1* Membrano proliferative glomerulonephritis type 1; IgA nephropathy, *OX* Oxalosis, *ATN* acute tubular necrosis, *CNI* calcinerurin inhibitors, *TX* transplant, *n/a* not applicable

less than 3 years will recur after kidney transplantation. Other risk factors include initial treatment resistance, initial steroid sensitivity with progression to steroid resistance, non-black race, and recurrence in previous transplants. The recurrence rate in a subsequent graft approaches 80% [32]. Notably, clinically significant disease recurrence does not necessarily affect all re-transplants following loss of the first graft to disease recurrence. Importantly, selected patients who experience first graft failure due to recurrent glomerular disease should not be precluded from receiving a second transplant [33]. Factors reported not to affect recurrence include HLA mismatch, histologic subtype, chronic dialysis duration, and type of immunosuppression.

Individuals with known genetic causes of FSGS do not appear to be at risk for recurrence in the transplanted graft. It is currently prudent to perform genetic testing as the presence of a genetic etiology portends a reduced recurrence risk, and if present could advocate for living donation with excellent outcomes [34]. Otherwise, in patients with primary FSGS, there is no evidence of the superior graft survival advantage seen in non-FSGS transplants in living-related donor over the deceased donor transplants [35]. Currently, deceased donor transplants are preferential in individuals with primary FSGS.

Recurrence can occur immediately after transplantation and result in massive proteinuria, hypoalbuminemia, acute tubular necrosis, and graft failure due to small vessel thrombosis. It remains the most common cause of graft loss in pediatric renal transplantation. Early on, foot process effacement is the only finding on biopsy, characteristic FSGS lesions may follow later in the course [36].

Management remains controversial. The current approach is often based on anecdotal evidence, derived from non-blinded, non-randomized, small case series. Recognizing the role of circulating factors in the pathogenesis, plasmapheresis is used prophylactically prior to transplantation or immediately after when there is evidence of recurrence disease. A recent study has shown that prophylactic plasmapheresis did not provide any additional benefit prior to the transplant in living and deceased donors did as regards the incidence (27% vs 26%, $P = 1.0$) or time to recurrence of FSGS in the kidney allograft ($P = 0.22$) [37]. This has been supported by others [38].

Prompt treatment of recurrence with these therapies may result in improved outcomes with or without rituximab [39].

Twenty to sixty percent remission rates have been reported in different centers at variable lengths of post-transplant plasmapheresis treatment [37]. Immunosuppression protocols using rituximab and calcineurin inhibitors have also suggested a beneficial effect, although these findings are quite variable. Rituximab appears to have non-B-cell related effects as it sustains the integrity of the podocyte actin cytoskeleton and prevents apoptosis [40]. Rituximab can be recommended as a treatment for cases that have failed either the initial plasmapheresis or weaning from plasmapheresis although its benefit has not been shown consistently [41]. Abatacept is a co-stimulatory inhibitor that targets B7–1. Patients with FSGS have been found to be positive for B7–1 immunostaining of podocytes in kidney-biopsy. Abatacept induced partial or complete remissions of proteinuria in some possibly by stabilizing the β1-integrin activation in podocytes and reducing proteinuria in patients with B7-1-positive glomerular disease [42]. However, this was not replicated by others [43] and this drug has not gained popularity as an effective treatment. LDL-apheresis in combination with steroids has also been shown to be a promising treatment of FSGS recurrence post-transplant [44] and is gaining traction in the nephrology community.

C3 Glomerulopathy (45%)

C3 glomerulopathy encompasses C3 glomerulonephritis (C3GN, previously membranoproliferative glomerulonephritis type III) and dense deposit disease (DDD, previously MPGN II). This set of diagnoses is characterized by the glomerular deposition of C3 in the absence of Ig deposition [45]. C3GN is an ultra-rare disease, only reported to be the primary diagnosis leading to ESKD in 0.8% of children (NAPRTCS). DDD is very rarely the cause of ESKD in children [19]. Histologic differences based on electron microscopy are as follows: DDD is characterized by dense osmiophilic intramembranous deposits and C3GN by light dense amorphous mesangial, paramesangial, subendothelial, and subepithelial deposits.

The predominance of C3 deposition within the glomerulus suggests the presence of dysregulated alternate complement cascade. Most frequently, dysregulation occurs at the level of C3 convertase of the alternative pathway in the fluid phase and is driven by genetic and/or acquired defects. Our understanding of the genetics of C3G is not yet comparable to that of aHUS. More data is required to inform genotype-genotype correlations.

Genetic mutations (Factor H, Factor I) or autoantibodies (e.g., C4 nephritic factor directed against C3 convertase or Factor H autoantibodies) resulting in dysfunctional complement regulatory proteins and therefore uncontrolled amplification of the C3 protein have been implicated [46] (Table 1). As in aHUS, all planned recipients of a living-related kidney should be screened for genetic abnormalities, and if one is present, the donor should be tested to exclude that genetic abnormality. The theoretical risk that donation may trigger disease onset must be discussed with the donor.

In contrast to the acute presentation of aHUS, with C3GN the disease follows a chronic indolent course. Acquired partial lipodystrophy and retinal drusen are reported and appear to be direct consequences of complement activation.

Recurrent disease (45%) is the major contributor to poor graft survival, with over 50% of patients with disease recurrence reported to experience allograft failure [47]. The timing and clinical presentations of patients with C3GN and DDD are different, with DDD more likely to recur later post-transplant and often associated with no clinical manifestations other than allograft dysfunction. C3GN presents with proteinuria, hematuria, hypertension, and declining glomerular filtration rate. Hypocomplementemia is commonly observed. Younger age at presentation and crescents on original biopsy have been associated with increased recurrence risk. Transplant glomerulopathy may be difficult to distinguish from recurrent C3GN since histological features may be very similar.

A specific treatment approach is not well established. All patients should target optimal blood pressure control below the 90th percentile or less than 120/80 mmHg. Priority agents include angiotensin receptor inhibitors (ACE) and angiotensin receptor blockers (ARB). Lipid control is also recommended. Data is insufficient to recommend eculizumab as a first-line agent, though it is starting to be used if there are identified mutations [48] (see Table 1). Studies have included small numbers and heterogeneous patients. Hopefully, with the new classification, it will lead to more personalized treatment strategies for those who develop recurrent C3 glomerulopathy post-transplant.

Membranous Nephropathy

Membranous nephropathy is the most common cause of nephrotic syndrome in older adults but is an uncommon condition in children. The disease is characterized by the presence of subepithelial immune deposits with expansion of the glomerular basement membrane, causing podocyte injury.

Recurrent membranous nephropathy ranges between 10% and 45%. The reason for the wide variability is that the diagnosis is made only by biopsy. The discovery of autoantibody against the podocyte phospholipase A2 receptor (PLA2R) led to better understanding, management, and treatment of patients with idiopathic membranous nephropathy [49].

Risk factors for disease recurrence include: high titer of anti-PLA2R antibody, rapid progression of native ESKD, and prior allograft failure secondary to recurrent membranous nephropathy (especially if it occurred less than 5 years post-transplant).

Recurrent membranous nephropathy is typically observed 13–15 months after transplantation, although may also be observed within weeks. Patients can present with proteinuria and allograft dysfunction or subclinical histological recurrence. Higher relative risk of allograft failure has been reported after disease recurrence.

Treatment typically includes a combination of anti-proteinuric agents, corticosteroids, rituximab, calcineurin inhibitors, and alkylating agents. Patients with no or minimal proteinuria, stable glomerular filtration rate and only histological evidence of recurrence can be treated with non-immunosuppressive therapy alone as ACE or intensive blood pressure control. With significant proteinuria, both non-immunosuppressive and immunosuppressive therapy should be used. With rituximab, 80% achieve complete or partial remission when used for early disease recurrence post-transplant [50]. Bortezomib has been prescribed for rituximab resistant cases [51].

MPGN Type 1

Membranoproliferative glomerulonephritis type I is the primary diagnosis in about 2% of pediatric kidney transplants [19]. The pathogenesis involves a dysregulation of the classical or lectin complement system, caused by increased amounts of circulating immune complexes due to chronic antigenemia (chronic infections, autoimmune diseases, malignancies, plasma cell dyscrasias, or other persistent antigen stimuli) leading to the activation of the classical or lectin pathway of complement. Therefore, they present with a concurrent low level of C3 and C4. Histologically, it is characterized by glomerular immunoglobulin deposition in the subendothelial glomerular space, giving rise to marked mesangial proliferation [52]. Clinically patients present with nephrotic, nephritic, or nephritic-nephrotic overlap syndromes.

Rate of recurrence is about 30% in adults and pediatrics, although it appears to be higher in pediatric patients than that of adult patients.

Recurrence is manifested by proteinuria. Despite the risk of recurrence, graft survival is comparable to that of patients with other causes of graft failure [53].

Treatment includes plasmapheresis, steroids, and other immunosuppressive agents. However, the optimal treatment strategy remains undetermined.

IgA Nephropathy

IgA nephropathy is an immune complex-mediated glomerulonephritis. It has been recognized worldwide, although prevalence varies from one country to another. Its clinical presentation is frequently associated with an upper respiratory tract or gastrointestinal infection and onset of gross hematuria. On biopsy, granular electron-dense deposits are observed in the glomerular mesangial area by electron microscopy and confirmed as containing IgA and C3 by immunofluorescence. The immunopathologic features are very similar to Henoch-Schonlein purpura nephritis.

Recurrent IgA nephropathy is common. Its presentation is often relatively benign and tends to occur late post-transplant. The majority of patients have a relatively benign presentation with microscopic hematuria or evidence of IgA deposition in allograft biopsies. Proteinuria or progressive graft dysfunction can also be seen. Graft lost is still reported after IgA recurrence but with better outcomes than other recurrent conditions. Markers for progression, as with all glomerulonephritis, are increased urinary protein excretion and increased sclerosis or fibrosis on biopsy.

Several risk factors for disease recurrence have been described including younger age, recipients of zero-HLA mismatched live-related donor kidneys (though other data suggests no association with full HLA match and risk of recurrence [54]), steroid-avoidance or early steroid-withdrawal immunosuppressive regimens, the non-use of induction therapy, HLA allelic subtypes, increased serum IgA concentration, crescentic (rapidly progressive) IgA nephropathy in the native kidneys [55]. Pre-transplant tonsillectomy does not appear to affect IgA nephropathy recurrence [56].

The optimal treatment of recurrent IgA nephropathy remains unknown. Maintaining or changing to a calcineurin-inhibitor and corticosteroids-based immunosuppressive regimen in addition to anti-proteinuric treatments is the current practice. ACE or ARB therapy may delay progression from recurrent disease in the allograft [57, 58].

A different scenario is when there is incidental finding of IgA from donor source. This has been mostly reported in the Japanese and Chinese literature. Presence of IgA on the graft biopsy has not been shown to have an adverse association with graft or patient survival [59].

Other Glomerulonephritis (GN)

Systemic Lupus Erythematosus (SLE). SLE is an episodic, multisystemic, autoimmune disease with polyclonal B cell activation, and widespread production of autoantibodies. There is widespread inflammation of blood vessels and connective tissue due to immune complex deposition. It is more common in females and in the black population. Renal disease is more severe in patients with childhood-onset lupus than those with adult-onset disease. The reported recurrent lupus nephritis varies between 0–40%, and those who experience recurrence have greater risk of allograft failure and mortality [60].

Anti-glomerular Basement Membrane (GBM) GN and Goodpasture's Syndrome Goodpasture is a “pulmonary-renal-syndrome” characterized by pulmonary hemorrhage and crescentic nephritis. It is an auto-immune complex mediated disease. The presence of anti-GBM antibodies in peripheral blood confirms the diagnosis. The incidence of recurrent linear immunoglobulin G (IgG) staining in the transplant may be as high as 50%. However, most patients remain asymptomatic. Allograft failure from disease recurrence is exceedingly rare in the modern era of immunosuppression [61].

Anti-neutrophilic Autoantibodies (ANCA) Vasculitides. ANCA associated vasculitides are a heterogeneous group of rare autoimmune conditions that causes an inflammation of blood vessels with various manifestations. It includes three main diseases which are granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA; previously known as Churg-Strauss syndrome), and microscopic polyangiitis (MPA). In patients with ANCA-associated vasculitis, the relapse rate (often extra-renal) has been reported at 0.02 per patient-years, with no consistency with disease severity prior to transplantation and recurrence post-transplantation [62].

Non-glomerular Causes

Primary Oxalurias

Primary hyperoxaluria type 1 (PH1) is an autosomal recessive disorder due to decreased activity of hepatic alanine-glyoxylate aminotransferase. This leads to over production of oxalate by the liver, increased urinary excretion of oxalate and glycollate and progressive kidney failure. Reduced clearance leads to deposition of oxalate in body tissue. Confirmation of diagnosis is by genetic analysis or enzyme assay on liver biopsy. Infantile type is severe, presenting with nephrocalcinosis and early ESKD. Crystals can be seen in the retina and bone marrow. The only treatment is removing the source of excess oxalate production via a liver transplant as early as possible. Up to 40% patients are pyridoxine sensitive. Other adjuvant treatments include large fluid intake and oral citrate. Hemodialysis provides better clearance than peritoneal dialysis.

In patients with primary hyperoxaluria, recurrence of oxalate deposits in the graft occurs universally and can lead to graft failure in the same way it led originally to ESKD. Combined or sequential liver-kidney transplant is the best approach to preventing systemic oxalosis. Transplantation of liver first, with intense dialysis before kidney transplantation could potentially lower the burden of oxalate in the body and decrease the likelihood of compromising the graft [63].

Summary

Disease recurrence is currently one of the most challenging issues in pediatric kidney transplantation. There have been significant advances in the understanding of the epidemiology, pathogenesis, and classification of recurrent diseases. Despite these advances, considerable uncertainty remains in the approach and treatment. Better understanding of disease pathophysiology, risk evaluation and individualized treatment strategies will improve morbidity and patients' outcomes. Genetic testing should be performed whenever available. It is imperative to establish global registries collecting comprehensive in-depth data of pediatric patients with recurrent disease, bio-repositories, as well as novel trials designed to test the efficacy of specific treatment strategies in multicenter randomized controlled trials to address the knowledge deficiency in this disease. Disease recurrence should not preclude future pediatric transplantation, it should rather urge to investigate further and establish personalized specific treatment strategies.

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Renal Transplantation: Infectious Complications

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Abstract

Infections are a major cause of morbidity and mortality after transplantation, not only in the immediate post-operative period but also beyond. The infectious burden in the post-transplant setting is mostly related to the iatrogenic immunosuppression required to avoid graft rejection, but also to other factors such as surgical site infection, cytopenias, and community exposures (Fishman, Am J Transplant

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F. Emma et al. (eds.), *Pediatric Nephrology*,
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17:856–79, 2017). In comparison to adults, pediatric kidney transplant recipients are at further risk for infections because they have not had the opportunity to encounter many pathogens and mount an immune response prior to transplant. This chapter focuses on frequently encountered bacterial, viral, fungal, and parasitic infections following pediatric kidney transplantation.

Keywords

Kidney transplantation · Donor-derived infection · Herpesviruses · BK virus · Urinary tract infection

Introduction

In the previous chapters the reader has become acquainted with the science of pediatric kidney transplantation. The reader will also have noted the tremendous successes achieved in this field over the past few decades, with marked improvement in patient and graft survival over the last decades [2–4]. Due to improved diagnostic techniques, clinicians' awareness, longer survival, and more potent immunosuppressive regimens, infectious complications such as post-transplant lymphoproliferative disorder (PTLD) and BK virus allograft nephropathy (BKVAN) are becoming more prominent and more frequent [5–7]. The infection-hospitalization cumulative incidence is much higher at 47% in children in the first 3 years post-kidney transplant than while on dialysis or in comparison to adults post-kidney transplant [8]. Unlike adults, the total cumulative infection incidence in children did not drop in recent years [9]. In this chapter we will discuss the various infectious complications associated with pediatric kidney transplantation (Table 1).

Special Considerations in Pediatric Transplantation

When compared to healthy individuals, organ transplant recipients of any age are at greater risk for infections since the currently used immunosuppressive agents also suppress the defense mechanisms against infections [10]. Besides the immunosuppressive agents, other factors contribute to the net state of immunosuppression, such as cytopenia, loss of integrity of the mucocutaneous barrier, underlying immunodeficiency, and post-operative complications [1]. In addition, *pediatric* recipients of organ transplants have some unique (usually additional) features that add to their risk for complications. By virtue of their younger age, these recipients are potentially exposed to disease complications and medication toxicities for a longer period of time. The risk for some infections, especially viral infections, is higher if the child has not been exposed to that organism while still immunocompetent. Thus, a primary infection in a host who is immunosuppressed tends to be more severe. The kidney allograft often acts as the source for the primary infection, since most children receive an adult donor kidney, which harbors latent viruses – especially herpesviruses and polyomaviruses – to which the donor has previously been exposed. Many children may not have had the opportunity to receive all vaccinations prior to transplant, thereby preventing development of immunity against vaccine-preventable diseases.

Table 1 Typical post-transplant infectious complications

Infectious
Bacterial
Wound infections: usually *Staphylococcus/ Streptococcus* spp
Central line infections
Pneumonia
Urinary tract infections: usually Gram-negative rods
Mycobacterium tuberculosis
Bartonella henselae
Viral infections
Cytomegalovirus
Herpes simplex virus
Epstein-Barr virus
Varicella virus
BK virus
Other
Pneumocystis jirovecii
Candida spp
Other fungi
Parasitic

Bacterial Infections

Urinary Tract Infection

Urinary tract infections (UTI) affect kidney transplant recipients more than any other type of transplant recipients [11]. UTIs are the most common bacterial infection in kidney transplant recipients, both in adults [12, 13] and children >5 years [14]. The percent of renal transplant recipients who will experience a UTI ranges from 20% to 65% in the first year post-transplant [12, 15] and 40–80% by 3 years post-transplant (Table 2) [16–20]. The highest incidence of UTI happens 3–6 months following kidney transplantation [11]. UTI is not only a cause for morbidity but is also associated with higher rates of graft loss and patient death [16, 21]. Early UTI (within 6 months of transplant) elevated the risk for graft loss in children, while late UTI did not [22]. The urogenital tract is the most common entry point for the development of sepsis [23], and UTI are responsible for 30% of sepsis episodes following kidney transplantation [11]. Risk factors for UTI in children include bladder abnormalities, the need for chronic or prolonged bladder catheterizations, and placement of a ureteral stent [17, 24]. In adults, diabetes mellitus and cardiovascular disease are comorbid factors that also associate with a higher incidence [16].

Table 2 Complication rates in multicenter randomized trials in pediatric kidney transplantation

Complication		Infections/malignancies		
		UTI	CMV	PTLD
Trompeter et al. (CsA vs Tac) [137]	Control arm	33%		2%
	Study arm	29%		2%
Grenda et al. (basiliximab addition) [138]	Control arm	28%	2%	2%
	Study arm	19%	7%	0%
Offner (Basiliximab vs placebo) [139]	Control arm	23%	9%	5%
	Study arm	35%	13%	2%
SNS01 study (steroid avoidance) [140]	Control arm	31%	9%	0%
	Study arm	25%	14%	0%
TWIST study (early steroid withdrawal) [141]	Control arm	13%	12%	0%
	Study arm	17%	12%	2%
Tonshoff et al. (everolimus versus MMF) [142]	Control arm	24%	6%	0%
	Study arm	25%	2%	2%

Footnote: The IVIST trial [143] compared the steering of immunosuppression based on virus-specific T-cell levels in the intervention arm to trough level immunosuppressive drug monitoring. Reduced levels or daily immunosuppressive doses in the intervention arm were associated with a trend to reduced EB viral replication and no adverse impact on allograft function or CMV replication

UTIs in kidney transplant recipients are usually classified as asymptomatic bacteriuria, acute simple cystitis (no systemic symptoms), and complicated UTI/pyelonephritis (systemic symptoms or indwelling stents, catheters or nephrostomy tubes) [11]. Recurrent UTI is defined as ≥3 episodes in 12 months [11].

The organisms implicated are usually the same as in immunocompetent individuals, mainly Enterobacteriaceaes such as *Escherichia coli*, followed by *Klebsiella* spp and *Proteus* spp. A higher percentage of UTIs in transplant patients are due to unusual organisms such as *Pseudomonas* spp and *S. saprophyticus* [11, 25]. Clinical symptoms include fever, dysuria, graft tenderness, and cloudy urine. In some patients, systemic symptoms such as fever may be masked by the immunosuppressive medications. A rise in serum creatinine often occurs and can mimic acute rejection. UTIs can also precipitate an acute rejection. The diagnosis of UTI is usually confirmed by urine culture, though patients on trimethoprim-sulfa prophylaxis for *Pneumocystis jirovecii* may not demonstrate positive cultures. In that case, radionuclide scanning or clinical judgment may be necessary. Recently, the emergence of multidrug-resistant organisms as a cause of UTI following kidney transplantation may be associated with poorer prognosis and an increased likelihood of recurrent UTIs [11]. Treatment is with antimicrobial agents. Empirically, betalactams or quinolones with coverage against common Gram-negative organisms should be used as a first -line therapy [11]. Once the organism is identified, the most specific and cost-effective antimicrobial can be prescribed. Treatment route and total duration vary somewhat according to the severity of the infection, recipient age, and other risk factors. Shorter oral 5–7 days courses such as used in immunocompetent people can be used for milder cystitis episodes in older children. In more severe acute pyelonephritis in a younger child who was recently transplanted, intravenous antimicrobial therapy for 14–21 days is preferred. Currently, the available evidence does not support routine treatment of asymptomatic bacteriuria [11].

Not all centers prescribe routine prophylaxis for UTI. Trimethoprim-sulfa, if given daily, may serve as both UTI and *Pneumocystis jirovecii* prophylaxis and is recommended in the KDIGO and AST guidelines [11, 26].

Other Bacterial Infections

Other bacterial infections, such as wound infections, line sepsis, and pneumonia, are seen with significant frequency in kidney transplant recipients. Wound infections and line sepsis are commonly due to Gram-positive *Staphylococcus* spp and *Streptococcus* spp. While many (44%) cases of pneumonia are bacterial in origin, pneumonia can also be due to viral or fungal organisms [27]. In adult transplant recipients, cellulitis and bacterial abscesses are frequent problems, largely due to co-morbid diabetes mellitus. The detection and treatment of these infections is generally no different than standard investigation and treatment in immunocompetent hosts, though duration of therapy may be longer.

Bartonella henselae infection (also known as cat-scratch disease) has been reported in pediatric organ transplant recipients, including kidney transplants [28]. This infection typically presents as fever and lymphadenopathy, thus becoming a differential diagnosis for PTLD. However, unlike PTLD, this infection is curable with antimicrobial therapy.

In developing countries, *Mycobacterium tuberculosis* infection occurs more commonly. The incidence is <2% in Western countries but ranges from 5% to 15% in Asian and African countries [29, 30]. Use of cyclosporine, co-existing diabetes mellitus, and chronic liver disease increase the risk for post-transplant tuberculosis [31]. Active tuberculosis infection can present with multiple symptoms, including weight loss, cough, fever and lymphadenopathy, again acting as a differential diagnosis for PTLD. However, pulmonary involvement is more common than lymphadenopathy in the post-transplant setting. Early diagnosis is best achieved by staining for acid-fast bacilli or DNA amplification by polymerase chain reaction (PCR) from sputum, bronchoalveolar lavage or gastric aspirates. A four-drug regimen, similar to the regimen recommended in the general population, should be used in case of active tuberculosis after transplantation [32]. Rifampicin can be part of the regimen but careful attention should be paid given its interaction with tacrolimus/cyclosporine/steroids, requiring to markedly increase the dose of immunosuppression. Alternatively, rifabutin could be used instead of rifampicin given its milder interactions [32].

Latent tuberculosis is defined by the evidence of exposure to the bacilli (either by a tuberculosis skin test [TST] or an interferon-gamma-release assay [IGRA]) in the absence of symptoms or radiologic abnormalities. When diagnosed after treatment, treatment with isoniazid for 9 months should be considered [32].

Viral Infections

Cytomegalovirus

Cytomegalovirus (CMV), now also called humanherpes virus (HHV)-5, is a double-stranded DNA virus of the herpesvirus family. In pediatric kidney transplantation, the incidence of CMV infection has been highly variable, ranging from 6% to 40% (Table 2) [33–36]. The variation is partly due to differing definitions (e.g., asymptomatic replication versus CMV syndrome versus CMV organ disease) and partly due to different detection methods [35, 37]. A retrospective study in pediatric transplant recipients using pp65 antigenemia reported a viremia rate of 21% and disease rate (viremia plus organ involvement) of

9.7% [35]. In pediatric kidney transplant recipients, incidence of CMV infection and disease were 40% and 15%, respectively [38]. CMV is especially important because of its dual effects: it causes significant morbidity by direct infection, yet its immunomodulatory effects can predispose to other infectious complications [39]. CMV infection post-transplant can be as a primary infection, a reactivation infection, or a superinfection. Of these, primary infection in the immunocompromised host is the most likely to lead to disease and often occurs when a seronegative pediatric recipient receives a seropositive kidney transplant [35, 36]. Both CMV viremia and disease usually occur within the first few months post-transplant, though routine antiviral prophylaxis has led to a shift, with more frequent late-onset CMV disease appearing after prophylaxis ends [37]. CMV infection may present as asymptomatic replication, CMV syndrome, characterized by fever, myalgias, malaise, leukopenia and thrombocytopenia, or CMV organ disease, in which there is clinical evidence of organ involvement such as pneumonitis or colitis [39, 40]. The transplanted kidney is at higher risk for CMV infection than the native organs are, but pulmonary, liver, and gastrointestinal tract involvement are common, regardless of the organ transplanted.

As stated above, in addition to causing direct infection, CMV has significant indirect effects including an increase in the overall state of immunosuppression leading to a greater risk for heterologous infections [41]. CMV infection also increases the risk of EBV-associated PTLD [42]. In addition, CMV and acute rejection are interrelated. Kranz et al. showed that a previous CMV infection is a risk factor for acute rejection, while rejection is known to release of tumor necrosis factor, triggering the process that ultimately leads to CMV replication [35].

Prevention of CMV infection can be accomplished with either (1) universal prophylaxis: the administration of anti-CMV therapy to all patients; (2) preemptive treatment: the systematic monitoring of CMV replication with administration of anti-CMV therapy if viral load reaches a certain threshold; or (3) a hybrid approach combining a short prophylaxis following by monitoring of CMV replication [39, 40]. There is some controversy as to the optimal strategy, as both methods have advantages and disadvantages. In one study of pediatric liver transplant recipients, preemptive therapy was superior to prophylaxis [43]. Most guidelines recommend universal prophylaxis for high risk patients (e.g., seronegative recipients of seropositive organ), with preemptive therapy reserved for patients at low or intermediate risk [39, 40]. Although several agents are available for prophylaxis, ganciclovir and valganciclovir have largely supplanted either oral aciclovir or CMV immune globulin (CMVIG) as the recommended regimen for CMV prevention. Oral ganciclovir has a poor bioavailability and must be taken in large doses three times daily. Valganciclovir has a superior bioavailability, with a pharmacokinetic profile similar to intravenous ganciclovir. The standard duration of prophylactic therapy is now 180 days in adults. Although evaluated in phase III trials, no CMV vaccine is currently available.

Detection of CMV DNA or RNA by polymerase chain reaction (PCR) has now supplanted the pp65 antigenemia assay. Serum and whole blood DNA correlate well in case of CMV [44] and now an international CMV standard is more widely used.

When CMV disease does occur, treatment with oral valganciclovir or intravenous ganciclovir is equally effective [45]. Oral valganciclovir is usually preferred over intravenous ganciclovir, unless in case of life-threatening infection [39, 40]. The optimal duration of treatment has not yet been defined, and current protocols recommend continuing treatment until the viral load becomes undetectable for at least two consecutive measures. Cidofovir and foscarnet are second-line agents to be considered in case of (suspicion of) resistance to (val)ganciclovir [39, 40]. Novel agents such as letermovir have not been well studied in the SOT setting and resistance may develop earlier than with other anti-CMV agents [46].

Epstein-Barr Virus and PTLD

Epstein-Barr virus (EBV; now also known as HHV-4) is another DNA herpesvirus that causes significant morbidity post-transplantation. Like the other herpesvirus CMV, this virus commonly infects immunocompetent people sometime in childhood and establishes a prolonged latency in reticuloendothelial cells. Thus, the patterns of infection are identical to CMV: primary infection (often from the graft of a seropositive donor), reactivation, or superinfection. Again, like CMV, the primary infection in an immunosuppressed transplant recipient is more virulent. Unlike CMV, EBV infection does not seem to have many indirect effects except for the development of PTLD, which is an uncontrolled proliferation of immune cells (typically B cells) in the absence of T-cell control because of post-transplant immunosuppression [7].

EBV infection can be asymptomatic or present as a nonspecific viral syndrome, similar to CMV infection [47]. Routine PCR monitoring suggests that the rate of EBV infection in EBV seronegative pediatric transplant recipients (as defined by systemic viremia) ranges from 50% to 80% [48–50].

EBV viremia, in contrast to PTLD, can be asymptomatic or associated with mild nonspecific viral symptoms. In the past, the diagnosis was made by documenting seroconversion (EBV IgM positive or significant rise in IgG titer). Unlike with CMV, in the case of EBV, testing can be performed against EB nuclear antigen or viral capsid. The former turns positive early, after recent infection and the latter in more distant infection. Currently, PCR amplification of viral DNA has supplanted serological assays for the diagnosis of active EBV infection [51]. The reader should note that PCR techniques for EBV DNA amplification vary greatly based on the type of sample and laboratory standards. Thus, PCR values from peripheral blood leucocytes and whole blood generally correlate with each other but not with PCR values from plasma [52, 53]. An international standard for EBV was introduced in 2013 but did not eliminate the variability in results from different laboratories [54]. The KDIGO guidelines recommend the following post-transplant EBV schedule for high-risk D+/R patients: once in the first week after transplant, at least monthly for the first 3–6 months, then at least every 3 months until the end of the first year with re-initiation of monitoring after treatment for acute rejection [55].

In pediatric cases especially, most PTLDs are due to EBV [47]. This condition is not a true malignancy since the immune system can regain control of B-cell proliferation if extrinsic immunosuppression is reduced and EBV-directed CD8+ T cells regain function.

PTLD incidence in pediatric kidney transplantation was previously low at <1%, but then climbed to more than 4% before a more recent drop (Table 2) [2, 56–59]. The main risk factors for PTLD after pediatric kidney transplantation is a D+/R-serostatus, even though the use of antilymphocyte globulin and young recipient age are also considered as risk factors [47]. PTLD can present clinically in many ways [59, 60]. Most often the patient has lymphadenopathy, fever, or symptoms related to pressure on internal organs from an internal lymphoid mass. Definitive diagnosis requires histological confirmation, though imaging can help with appropriate tissue acquisition and staging. PTLD are categorized in different forms or early lesions, polymorphic lesions, monomorphic lesions, or Hodgkin-like lymphomas according the 2016 WHO classification [61]. Monomorphic PTLD is generally more severe than polymorphic.

Due to an absence of evidence of benefit, antivirals, IVIG, and rituximab are not routinely recommended for the prevention of EBV disease [47]. EBV vaccines have not been successful enough so far for clinical implementation.

There is no universally accepted treatment for EBV infection post-transplant [47]. Reduction in immunosuppression is one modality. The antiviral agents ganciclovir and valganciclovir have activity against EBV even though their clinical benefit is still debated. Aciclovir is not effective against EBV. The duration and route of antiviral treatment is not standardized but some centers will treat until the EBV PCR has turned negative. Whether all patients with acute EBV seroconversion need reduction in immunosuppression or antiviral treatment is a burning question in pediatric transplantation [47, 62, 63]. Children in particular can develop a chronic high load carrier state without ever progressing to PTLD [64]. Nevertheless, high EBV DNAemia has a good negative predictive value but a poor positive predictive value for the diagnosis of PTLD [47, 65–67].

Treatment of PTLD usually involves a sequential process [68, 69]. Reduction of immunosuppression reduction is typically the first intervention. Antivirals and/or IVIG are not recommended in the absence of other intervention [47]. If reduction of immunosuppression is not sufficient, the anti-CD20 antibody rituximab should be used in CD20 + B-cell PTLD. Chemotherapy is usually reserved for higher grade cases, such as progressive CD20+ PTLD despite rituximab or CD20- PTLD [47]. Rituximab can be added to either full dose or attenuated dose chemotherapy with good results with either type of combination therapy [70, 71]. Surgery and radiotherapy can be considered in selected cases. EBV-specific T-cell infusions are being investigated as a treatment for EBV + PTLD.

Varicella Zoster-Virus

Varicella-zoster virus (VZV), now also known as HHV-3, is the most infectious of the human herpesviruses. Primary infection with VZV results in chickenpox. Following primary infection, the virus remains in the body in a latent state from which it may be reactivated, resulting in cutaneous herpes zoster, or shingles. Incidence of any type of VZV infection in adult kidney transplant recipients was 7.4% in one study [72]. Children are more likely to be VZV seronegative at the time of transplantation and primary infection is a significant cause of morbidity and mortality [73, 74]. Routine VZV vaccination has been documented to reduce the incidence of primary VZV infection post-transplantation [75]. It is recommended that all transplant candidates over 9–12 months of age receive immunization with the VZV vaccine [76]. Studies in children with chronic kidney disease and on dialysis suggest that two doses, rather than one, may be necessary to elicit protective antibody levels. Therefore, antibody levels be obtained at least 4 weeks following immunization, and a second dose be given if necessary [76, 77]. Even though the American Academy of Pediatrics Committee on Infectious Diseases and the Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) advise against the use of this live vaccine in immunocompromised patients, accumulating data show that it could be safely administered in carefully selected SOT recipients [78, 79]. Hence, criteria for safe live vaccine administration after SOT have been published [80]. Where possible however, such vaccination should thus be provided, and protective antibody levels documented, prior to transplant [81–83].

Antiviral prophylaxis is not routinely recommended. First, patients receiving CMV prophylaxis will be indirectly prophylaxed against VZV given the efficacy of (val)ganciclovir on VZV. For patients not receiving CMV prophylaxis nor HSV prophylaxis, short-term prophylaxis against VZV using (val)aciclovir is recommended in VZV-seropositive patients [84].

Varicella-näive patients at the time of transplant (not immunized or did not respond to immunization) exposed to varicella should receive prophylactic therapy with varicella zoster immune globulin (VariZIG). The ACIP recommends that use of this product may be requested in immunocompromised patients exposed to varicella infection [85]. If this product is not available, intravenous immune globulin (IVIG), which contains some anti-varicella antibody, may be given [85]. Any prophylactic therapy should be given as soon as possible, up to 10 days after exposure [86]. Prophylactic aciclovir may be used after exposure, especially if the window for VariZIG has passed, though data are limited [86]. Patients who develop infection, either primary or secondary, should receive treatment with intravenous aciclovir [84, 87, 88]. Once lesions are improving, oral valaciclovir can be considered. In case of a mild localized (demartomal) zoster, initial treatment with oral valaciclovir can be considered [84].

BK Virus

The BK virus (BKV) was first isolated from the urine of a renal transplant recipient in the 1970s [89]. However, this virus did not represent a significant complication of kidney transplantation until the late 1990s [90, 91]. Though BKV is a polyoma virus, it shares some characteristics of the herpesviruses: (a) infecting most immunocompetent people during childhood; and (b) establishing a prolonged latency. Unlike the herpesviruses, the BK virus does not establish in the reticuloendothelial cells but prefers the uroepithelium. This propensity for the uroepithelium is responsible for the clinical manifestations: hemorrhagic cystitis in bone marrow transplant recipients and allograft nephropathy in kidney transplant recipients. The incidence of BKVAN in pediatric renal transplantation appears to be the same as in adult kidney transplants at 3–8% [5, 92]. Risk factors from adult kidney transplant data include the intensity of immunosuppression [93, 94], recent treatment for acute rejection [95] and placement of a ureteral stent [96], though the data implicating specific immunosuppressive agents is conflicting. In a multicenter North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) special study, use of antibody induction and zero mismatch kidney allografts were the only two risk factors for BKVAN in multivariate analysis [5].

BKV infection and BKV disease are two separate entities. Serial PCR surveillance has shown that urine PCRs turn positive earlier and much more often than peripheral blood PCR. The diagnosis of BKVAN depends on demonstration of the virus in blood or kidney tissue (either by PCR or immunostaining) and the presence of nephropathy (raised serum creatinine or tissue inflammation). Urine positivity without the other features represents infection without disease, as discussed in the recent guidelines for study monitoring by the American Society of Transplantation [97]. Generally, isolated viruria is not associated with renal dysfunction [98].

BKVAN represents a diagnostic challenge because the condition may resemble acute rejection. Symptoms are often minimal or absent. Serum creatinine elevations are found on clinical laboratory monitoring. Since the treatment of acute rejection (intensifying immunosuppression) is the opposite of the treatment of BKVAN (reduction in immunosuppression), making the correct diagnosis is critical.

Isolated BK viremia without nephropathy may not need treatment or may be manageable by reduction in immunosuppression. Like the CMV and EBV infections, reduction of immunosuppression is usually the first step in the treatment of full-fledged BKVAN [91, 99, 100]. However, by the time BKVAN is diagnosed, the serum creatinine is significantly raised and subsequent graft survival is worse. Antiviral therapy against BK virus is more complicated than for CMV or EBV, since neither aciclovir, ganciclovir, nor their analogues have activity against BK virus. Therefore many centers have tried alternative agents, given the impact of BKVAN upon allograft survival [101–105]. So far, no randomized studies have proven the benefit of either leflunomide, cidofovir, fluoroquinolones, or IVIG; consequently, these agents are not routinely recommended [97].

There is no vaccine against BK virus. Preventive strategies that have been suggested include serial urine or plasma BK PCR monitoring and preemptive reduction in immunosuppression. For all kidney transplant recipients, BK virus guidelines recommend monthly plasma monitoring until 9 months post-SOT, followed by monitoring every 3 months [26, 97].

Adenovirus

Adenovirus infections are common after kidney transplantation, with reported incidences of 4–7% in adults and 11% in children [106]. Clinical presentation can range from asymptomatic replication to organ disease such as hemorrhagic cystitis, hepatitis, pneumonia, or colitis [106]. Adenovirus infection frequently involves the graft, with graft dysfunction regularly reported. In case of adenovirus disease, the mainstay of the management is reduction of immunosuppression. For severe or disseminated disease not responding to reduction of immunosuppression, intravenous cidofovir should be considered despite the absence of randomized studies demonstrating its benefit [106].

Common Respiratory Viruses

Respiratory viruses are responsible for significant morbidity and mortality following pediatric transplantation. Despite an increased severity among transplant recipients, clinical presentation is usually similar to the general population [107].

Influenza virus infection is one of the most common respiratory viral infection and the only one for which an effective preventive strategy is widely available. Indeed, all transplant recipient should receive the inactivated tri- or quadrivalent influenza vaccine yearly [107]. In case of suspected or documented influenza infection, treatment with a neuraminidase inhibitor such as oseltamivir or zanamivir should be administered as soon as possible given the fact that early treatment has been associated with improved outcomes [107].

Other clinically relevant viruses in kidney transplantation are respiratory syncytial virus, parainfluenza virus, and human metapneumovirus. Unfortunately, no effective prevention strategies nor efficient treatments are available, even though ribavirin could be considered in specific situations [107].

SARS-CoV-2

The current pandemic has severely affected adult kidney transplant recipients, with fatality rates up to 28% [108]. This is higher than in recipients of other organs [109, 110] and likely related to the vascular comorbidities classically seen in kidney transplanted adults. So far, only a few cases of COVID-19 have been reported in pediatric kidney transplant recipients [109, 111, 112], and all had a relatively mild presentation, as seen in healthy children. It has been suggested that the immunosuppressive treatment may protect against severe SARS-CoV-2 infection by avoiding the hyperinflammatory state [113]. A document providing expert opinion regarding prevention, diagnosis, and management of SARS-CoV-2 infection among pediatric SOT candidates and recipients has been recently published [114].

Fungal Infections

In the SOT setting, the one-year cumulative incidence of invasive fungal infection (IFI) is around 3% [115]. However, with a one-year incidence of 1%, kidney transplant recipients are at lowest risk of IFI when compared to the other organ recipients [115]. Similarly, in the pediatric setting, IFIs are less common in pediatric kidney transplant recipients than thoracic organ recipients. Two studies reported a retrospective incidence of 4–10% overall (including milder *Candida* spp infections) in adult kidney transplant recipients [20, 116]. In studies restricted to the intensive care unit, the incidence is higher at 16.5% [117]. Among pediatric recipients, hospitalization for fungal infection represented 0.2–2.7% of all infection-related hospitalization in the NAPRTCS registry, but was associated with a 1.64-fold higher risk for graft loss [118].

Candida spp

Candida spp are the most common fungal organisms affecting kidney transplant recipients, either as oral thrush, vaginitis, nail infection, UTI, or rarely as systemic fungemia [119]. The diagnosis of thrush is made by clinical examination whereas candidal UTI is diagnosed by urine culture. Treatment for topical candida is by topical nystatin or clotrimazole and UTI is generally treated with fluconazole [120]. Invasive candida infection is responsible for 49% of IFIs in kidney transplantation [115] and is associated with significant mortality and morbidity [117]. Among *Candida* spp, *C. albicans* is most frequently responsible for IFI (42%), followed by *C. glabrata* (28%) and then other *Candida* spp. Treatment of Candida IFI usually with an echinocandin, with fluconazole and amphotericin being alternative options [120]. Given the overall low risk of Candida IFI after kidney transplantation, prophylaxis is generally performed for the first transplant month only by many centers. More prolonged prophylaxis may be considered in case of enteric drainage, vascular thrombosis, or postperfusion pancreatitis [120].

Aspergillus spp

Invasive aspergillosis (IA) is responsible for 14% of IFIs after kidney transplantation [115] and is reported in 1–4% of kidney transplant recipients [121]. Among *Aspergillus* spp, *A. fumigatus* is responsible for 75% of IA episodes documented after transplantation [121]. Moreover, about 75% of IA episodes are limited to the respiratory tract [121]. Risk factors for IA after kidney transplantation are a recent episode of acute rejection, graft failure, and dose and duration of exposure to steroids [121]. Voriconazole is the mainstay of IA's treatment. Anti-mold prophylaxis is currently not routinely recommended in kidney transplant recipients [121].

Pneumocystis

Infections with *Pneumocystis jirovecii* (previously known as *Pneumocystis carinii*) have fortunately become rarer with the widespread use of cotrimoxazole prophylaxis in the immediate post-transplant period. The diagnosis is established by demonstration of pneumocystis in lung secretions obtained from bronchoalveolar lavage or in tissue from lung [122]. Gomori stain or toluidine blue staining will demonstrate the cysts and Giemsa staining will identify the sporozoites. CMV infection is the major differential diagnosis. Many children may have dual infection, in which CMV infection predisposed to superadded pneumocystis pneumonia (PCP) infection. The antimicrobial agent cotrimoxazole (trimethoprim-sulfa) is the preferred treatment, with or without adjunctive steroids. Cotrimoxazole may be given either orally or intravenously and is less toxic, whereas the alternative pentamidine is only available as a parenteral or inhaled preparation and has more side effects. Chemoprophylaxis with three times a week oral cotrimoxazole (5 mg/kg trimethoprim component/dose) reduced the incidence of PCP disease from 3.7% to 0% [123]. Routine prophylaxis is recommended in all transplant recipients during the periods of high risk, typically the first 6–12 months post-transplant [124]. Some centers also advocate its use after anti-rejection therapy, particularly with anti-T cell antibodies. Some centers suggest a lower dose given daily at bedtime [125].

Parasitic Infections

Reports of parasitic infections in pediatric kidney recipients are few. *Strongyloides stercoralis* is an intestinal nematode with worldwide distribution, endemic in tropical/sub-tropical regions, and infects tens of millions of people. The southeast part has the highest incidence within the United States [126]. Similar to viruses, *S. stercoralis* may initially remain in the human intestinal tract without symptoms, and then disseminate with the introduction of immunosuppressive medication post-transplant [126, 127]. In addition strongyloidiasis transmission has been reported through the allograft kidney in an adult recipient [128]. Among calcineuirn inhibitors, cyclosporine (but not tacrolimus) has effects against *S. stercoralis* and may reduce the risk for disseminated strongyloidiasis [129, 130]. Active infection manifests with cutaneous, gastrointestinal and pulmonary symptoms as well as eosinophilia [131]. Fever, hypotension, and central nervous system symptoms suggest dissemination [131]. In uncomplicated infections, diagnosis is made by detection of larvae in stool, although 25% of infected patients may have negative stool examinations [132]. In disseminated disease, larvae may be found in stool, sputum, bronchoalveolar lavage fluid, and peritoneal and pleural fluid [126, 133]. Serologic testing using ELISA may also be of value, although may be falsely negative in immunocompromised hosts [133]. Ivermectin has replaced thiabendazole as the treatment of choice for *S. stercoralis,* with albendazole as an alternative [134].

Other parasitic infections that have been transmitted by the renal allograft include Chagas' disease [135] and post-transplant malaria [136]. Chagas' disease (caused by *Trypanosoma cruzi*) is found only in the southern United States, Mexico, and Central and South America. The manifestations of Chagas' disease classically include megaesophagus, megacolon, and cardiac disease, although CNS involvement has been reported in kidney transplant recipients [135]. The diagnosis is serological and treatment typically consists of benznidazole or nifurtimox. Post-transplant malaria can be frequent in high-endemic areas, so suspicion clinically should be based on local region or travel to such a region [136]. Screening recipients and donors for these and other parasitic infections may be worthwhile, based on the presence of risk factors including residence in or travel to an endemic area (Tables 1 and 2).

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Renal Transplantation: Non-infectious Complications and Long-Term Outcome

Christina Nguyen and Jaap Groothoff

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Abstract

Kidney transplantation is the best option for renal replacement therapy in nearly all children, but it comes with short- and long-term complications. Noninfectious transplant-related comorbidities largely originate from the specific side effects of immunosuppressive therapy and chronic kidney disease (CKD)-related problems caused by impaired graft function. Recurrent cramps from hypomagnesemia, hypertension, anemia, persistent growth retardation, tremors, cosmetic changes, and gastrointestinal problems are among the most common short-term side effects. Urological problems often occur and may severely impact graft survival. Calcineurin inhibitors-induced

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posterior reversible encephalopathy syndrome and post-transplant lymphoproliferative disease (PTLD) are rare, but may cause severe short-term complications. Post-transplant lymphoproliferative disorder (PTLD) is more prevalent in pediatric than in adult graft recipients. Post-transplant diabetes and dyslipidemia are less prevalent than in adults. Quality of life improves after transplantation coming from chronic dialysis treatment, according to most, but not all reports. Cognitive dysfunction is a common feature and may have an important impact on the quality of life.

Late complications into adulthood include a high risk for malignancies, especially for severe forms of squamous cell skin carcinoma. Additionally, cardiovascular disease, chronic joint pain and motor disabilities, and chronic fatigue. Cognitive impairment and delayed autonomy are associated social problems concerning sexual relationships, obtaining a paid profession, and starting a family. Despite these problems, long-term survivors of kidney transplantation consider their quality of life on average as good, especially with respect to mental domains.

Keywords

Non-infectious · Complications · Late outcome

Introduction

]Kidney transplantation is by far the best therapeutic option for children with end stage kidney disease (ESKD), but it is not curative of the disease. It comes with its own problems, induced by side effects of immunosuppressive therapy and the often limited graft function. Infection is the most common and life-threatening comorbidity, and is separately discussed in ► Chap. 83, "Renal Transplantation: Infectious Complications". Also, surgical complications, recurrence of primary disease, and chronic rejection are discussed elsewhere. In this chapter, we will first discuss the impact of pediatric kidney transplantation on somatic comorbidities other than infection, including cognitive development and psychosocial functioning in childhood. Finally, we will review what is currently known about late outcomes of pediatric kidney transplant recipients and the perspectives of transplanted patients in childhood on adult life with respect to somatic condition, related disease, and psychosocial functioning.

Short- and Medium-Term Complications

Introduction. Cramps by hypomagnesemia, most often related to the use of calcineurin inhibitors (CNI), chronic fatigue, and MMF-related gastrointestinal symptoms (pain, nausea, diarrhea) are the most often reported complaints after transplantation. Calcineurin inhibitor-induced tremors and headaches occur more rarely, but can be seriously disabling. Table 1 summarizes frequent and less common noninfectious somatic problems that are associated with kidney transplantation and its therapy. In this part, we will discuss in more detail the most important comorbidities and psychosocial consequences of kidney transplantation in children.

Hypertension

Epidemiology. Hypertension (HTN) is probably the most important modifiable risk factor for the development of life-threatening cardiovascular disease (CVD) in CKD5. As CVD is the second most common cause of death after kidney transplantation, early and maintained management of HTN is of utmost important [1]. Like in adults, HTN is also associated with shortened graft survival [1–4]. In one study, there was a two-fold higher risk of graft loss for each 10% increase in systolic blood pressure (BP) at 1 year after transplantation [4].

Yet, data on current practice show that control of HTN remains a challenge, not only in dialysis but also after kidney transplantation. Both US and European Registry studies show that uncontrolled

Table 1 Frequent early onset comorbidity of kidney transplantation in children

Complication		Frequency	Association	Impact
Cardiovascular disease	Hypertension	High	Pred, CI, graft dysfunction	Early graft loss, early mortality, impaired QOL
	Dyslipidemia	Moderate	Pred, mTORi	
	Obesity/metabolic syndrome	High	Pred	
	Post-tx diabetes mellitus	Low	CI + pred[a]	
Bone disease	Osteoporosis	High	Pred, immobility	Fractures, pain, motor disability, psychosocial impact of short stature
	CKD-MBD	Moderate	Graft dysfunction[b]	
	Growth retardation	High	Graft dysfunction	
Neurologic disorders	Headache	Moderate	CNI (TAC > CsA)	Disabling, psychosocial impact, impaired QOL
	Tremor	High	CNI	
	Convulsions	Low	CNI	
	Cramps	High	CNI, hypomagnesemia	
	PRES	Low	CNI	
Gastrointestinal disorders	Abdominal pain	Moderate	MMF	QOL
	Nausea			
	Diarrhea			
Hematologic disorders	Anemia	High	MMF, graft dysfunction	Quality of life
	PTLD	Low	EBV (CMV)	Mortality, early graft loss
	TMA	Low	CNI	Early graft loss
	Bone marrow depression	Moderate	MMF, CNI, mTORi	Infection
	Disturbed wound healing	Low/ moderate	Pred, mTORi	Perioperative complications, wound dehiscence
Cosmetic	Overt Cushing	Moderate–high	Pred	Non-adherence, graft loss, impaired QOL
	Hypertrichosis		CsA	
	Gingiva hypertrophy		CsA	
	Alopecia		TAC	
	Scarring		Surgery, pred	
Urinary tract disorders	Nephrotoxicity	High	CNI	Early graft loss
	VUR	High	Surgery	Urinary tract infection
	Dysfunctional voiding	Moderate	Bladder dysfunction	Urinary tract infection
	Ureteral stenosis	Low	Impaired graft function	Early graft loss
Psychosocial disorders	Impaired QOL	Moderate	Uncertain	Impact social life, impaired autonomy development
	Cognitive disorders	High	Uncertain	

CNI calcineurin inhibitors (cyclosporine/tacrolimus), *mTORi* mTOR inhibitors (sirolimus, everolimus), *Pred* prednisone, *MMF* Mycophenolate mofetil, *CsA* cyclosporine A, *TAC* tacrolimus

[a] High in case of steroid/CsA maintenance regimen[e]

[b] Impaired Ca/Pi/PTH management

HTN is highly prevalent in dialysis patients, but also persists in 21% of patients after kidney transplantation [5, 6]. Less than 30% of the transplanted patients in the ESPN registry had a normal BP without antihypertensive medication and more than 50% had BPs higher than the 75th percentile [5]. A UK registry study even showed an increase in uncontrolled HTN prevalence from 28% at time of transplantation to 50% after 2 years of follow-up [7]. A Dutch study found 38.3% uncontrolled HTN after kidney transplantation in patients aged <19 years and nearly 76% in patients aged 18–30 years [8]. HTN is probably underreported in these studies, as the prevalence of masked HTN – HTN found by 24-h ambulatory BP monitoring (ABPM) but not by office BP measurement – is higher among pediatric transplant recipients than found in any other pediatric cohort. Masked HTN may occur in up to 38% of patients and the majority of transplanted children have no adequate nocturnal dipping [9]. Data of the 4C study showed that nocturnal HTN was associated with an increased risk of subclinical CVD in children, as measured by surrogate outcomes markers [10].

Causes. There are three main sources of transplantation-related factors that may lead to HTN: (1) condition of the donor graft, (2) immunosuppressive therapy, and (3) de novo graft injury. Donor-associated factors include older donor age, small donor kidney size, preexisting donor HTN, and, above all, poor allograft quality [11]. Donor quality has been long recognized to be a significant contributor to post-transplant HTN. Recipients of the so-called extended criteria donors are especially at risk known for post-transplant HTN [12].

Transplant renal artery stenosis (TRAS) is a relative rare, but important cause of HTN after kidney transplantation. In adults, TRAS affects long-term graft survival, independent from the HTN that is concurrently induced [13]. Data in children are scarce, but the reported incidence of about 4–6% seems in line with adult reports [14–17]. In general, transplant renal artery stenosis should be considered in case of severe, therapy resistant HTN. In an Italian study of 10 cases among 216 pediatric kidney graft recipients, patients with renal artery stenosis required on average more antihypertensive medication than those with HTN for other reasons. Four out of ten had vascular abnormalities or complications during surgery. All were detected by Doppler ultrasonography [16]. Both delayed graft function or long ischemia time and cytomegalovirus (CMV) infection have found to be predictive for TRAS in adults [13].

Some genetic variants, either of the recipient or the donor are associated with subsequent post-transplant HTN. The role of variations in the apolipoprotein L1 gene (*APOL1*), as often is found in African-Americans, is complex with respect to post-transplant HTN. There is evidence that donors with two specific APOL1 gene variations in donor kidneys strongly predispose to the development of nephropathy, also post-transplant and subsequently to early graft loss and concurrent HTN, secondary to the nephropathy [18]. This particular gene variation is a stronger risk factor for early graft failure and parallel occurring HTN than any other known risk factor such as HLSA mismatch, expanded donation criteria, long cold ischemia time, or positive PRA [19]. The fact that African-American donor kidneys with less than two *APOL1* risk variants have equal outcomes to that of kidneys from European American donors shows that race per se is not the driving factor.

Steroids and calcineurin inhibitors are the most responsible immunosuppressive drugs for HTN. Cyclosporine induces sodium retention most likely as a result of afferent glomerular arteriolar vasoconstriction. Tacrolimus also leads to salt retention, but may be less associated with HTN than cyclosporine. Acute and chronic rejection and TMA are most common factors leading to de novo or worsening of existing HTN. Younger age, obesity, male gender, and high salt intake are the most important recipient-related factors contributing to HTN [20].

Management. Unfortunately, the evidence that adequate BP control is indeed beneficial for transplanted children with respect to graft survival and cardiovascular protection is limited. Seeman et al. showed in a prospective interventional study with intention to treat analysis that aggressive management of HT was associated with significantly less loss of estimated glomerular filtration rate (eGFR) after 2 years of observation [21]. However, in the ESCORT trial, no benefit was found from intensified BP control with respect to graft survival [22, 23]. The ESCAPE study showed improvement in surrogate outcome markers for CVD with angiotensin-converting enzyme inhibitors (ACEi) use in hypertensive children with CKD 2–4 [23], but only one small study showed similar results in transplanted children. Despite the paucity of evidence, Kidney Disease: Improving Global Outcomes (KDIGO) guidelines advise maintaining BP at below the 90th percentile for age, height, and gender (evidence level 2C) [24]. Steroid removal or reduction, in case of maintenance steroid immunosuppressive regimens, should be considered the first step. Salt restriction and prevention of obesity are also extremely important first-line measures. In the early post-transplant period, volume and salt overload often dominate, in which case a diuretic is the drug of choice. It is generally recommended that ACEi are avoided in the early period after transplantation due to its acute impact on eGFR and risk for hyperkalemia. In the longer run, calcium channel blockers (CCBs) and ACEi are currently the most used antihypertensive drugs after kidney transplantation. The preferable choice is under debate, but most meta-analyses recommend CCBs as first choice due to their positive effect on graft function and survival and lack of hyperkalemia and anemia as side effects. The presence of overt proteinuria might be a reason to choose ACEi as first choice. As HTN control can be difficult and several studies have shown that combining two or three anti hypertensive drugs can be very effective, a combination of CCB and ACEi could be a good solution for more severe HTN after transplantation. With the prescription of ACEi or ARB, the enhanced effect of CNI, mammalian target of rapamycin (mTOR) inhibitors, and cotrimoxazole on the hyperkalemic side effect – especially if eGFR declines – must be taken into consideration. There is increasing evidence that, especially in transplanted children, ABPM has an important role in monitoring adequate BP control, even more than in adults.

PTA is in over 80% of cases effective in reducing the BP in patients with transplant renal artery stenosis [13, 16]. Contrary to the non-transplanted situation, there is no consensus in the literature about the degree of arterial narrowing that would justify angioplasty, but generally, lesions causing arterial diameter stenosis of >50% assessed by angiography are considered to be hemodynamically relevant [16].

Dyslipidemia

Dyslipidemia is less frequent in pediatric transplanted patients than in dialysis patients, but may still contribute in many to cardiovascular morbidity and mortality. Among transplant recipients ($N = 564$) in the ESPN registry, 55.5% had some form of dyslipidemia, based on levels defined as “too high” by an expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents [25–27]. Eighty-eight percent of transplant recipients in the Certain registry had dyslipidemia defined as either “borderline high” or “high at 1 year post-transplantation [28].

Cyclosporine, steroids, and, most of all, mTORi have a relatively unfavorable profile, compared to tacrolimus (TAC) and mycophenolate mofetil (MPA) [25, 27, 28]. Regimens with cyclosporine, steroids, and mycophenolic acid (MPA) are associated with a 3- and 25-fold ($P < 0.001$) increased risk of having more than one pathologic lipid parameter as compared to the use of TAC, MPA, and steroids [28]. In a meta-analysis of adult kidney transplant recipients, the use of sirolimus was associated with a higher risk for dyslipidemia than any calcineurin inhibitor [29].

Post-transplant dyslipidemia may occur in various types. The use of cyclosporine instead of TAC may result in higher triglyceride and non-HDL levels in children as well as in adults [28, 30]. Steroid use is associated with higher triglyceride and non-HDL levels, the use of mTORi predominantly with higher non-HDL levels. In adult literature, no difference in lipid profile was found between the use of azathioprine versus MPA. The data in the pediatric literature are inconclusive [28, 30]. Other risk factors are a low eGFR and post-transplant diabetes mellitus. Despite the potential added cardiovascular risk, statin therapy is not recommended by KDIGO as hard evidence for its benefit and long-term safety is lacking. Adjustment of immunosuppressive therapy (cessation of steroids, avoidance of cyclosporine) and moreover nutritional counseling and lifestyle improvement are the most important interventions recommended [31].

Post-Transplantation Lymphoproliferative Disorder (PTLD)

The term post-transplantation lymphoproliferative disorder (PTLD) represents different types of lymphoid neoplasms that may develop after kidney organ transplantation. The World Health Organization (WHO) divides PTLD into four subtypes: an early, a polymorphic, a monomorphic, and a classical Hodgkin-lymphoma-like type (Table 1), each with its different therapeutic approach. PTLD may occur at any time after transplantation, but the highest incidence is within the first year after transplantation. These so-called early onset PTLD types are for 95% associated with Epstein-Barr virus (EBV) and have a relatively good prognosis [32].

Epidemiology and prognosis. PTLD complicates 0.8–20% of transplant recipients, about 0.8–2.8% in kidney transplant recipients [32–34]. The incidence is higher in patients receiving heart, lung, small bowel transplant, or combined heart-lung transplant than in kidney transplant recipients, which reflects the impact of the immunesuppression dosage [32]. Donor EBV-seropositive/recipient EBV-seronegative status, duration of immunosuppression, and status of EBV infection are the most important risk factors. Early-onset PTLD mostly occurs in the setting of EBV seropositivity and reactivation from latent B-cells as a consequence of immunosuppressive therapy (Table 2). The situation of an EBV-seronegative

Table 2 Late complications and potential preventive measures

Complication		Cause	Prevention
	Cardiovascular disease & mortality	Graft dysfunction, hypertension, dyslipidemia, dialysis years, calcineurin inhibitors	Vigorous control of blood pressure & dyslipidemia
	Motor disabilities	CKD-MBD, steroids, immobilization	Avoiding steroid maintenance therapy Ca/Pi/PTH/Vit. D management Consider bisphosphonates
	Cancer	Immunosuppressive therapy, UV, viral infection (EBV, papilloma)	Sun protection Papilloma vaccination (?) Vigorous EBV monitoring Tailoring of immunosuppressive therapy
	Infections	Immunosuppressive therapy	Tailoring immunosuppressive therapy, pre-tx vaccination
	Delayed autonomy development	Social deprivation by hospitalization and overprotection, cognitive impairment, physical strain	Active management toward autonomy at adolescence Avoidance of “overprotection”

recipient getting an allograft from an EBV-seropositive donor (D+/R−) may lead to a primary EBV infection, which is the most important risk factor for developing PTLD. This is why PTLD occurs two to eight times more often in pediatric than adult transplant recipients [35, 36]. The time from transplantation to PTLD diagnosis ranges from 6 months to 7 years and shows a biphasic pattern [36]. Late onset PTLD is most often not related to EBV but to the impact of relative high burden of immunosuppressive therapy and has an overall worse prognosis than the early onset form [36, 37].

Pathophysiology of EBV-associated PTLD. EBV is a lymphotropic virus which enters the B-lymphocytes during the initial infection and remains in the host nucleus in a state of latency for that individual's lifetime. This latent state is associated with EB nuclear antigen production and Latent Membrane Proteins, which protect the B-cell from apoptosis and stimulate ongoing viral replication that is controlled by a cytotoxic T-cell-driven EB-specific surveillance. Impairment of this cytotoxic T-cell protection is believed to be essential for developing PTLD [38]. Lack of T-cell protection leads to uncontrolled viral replication and an increase of virus-carrying B-cells in the circulation [38]. Cytokine responses, induced by immunosuppression, may lead to uncontrolled B-cell proliferation and ultimately neoplastic PTLD.

Monitoring and early detection. Elevated levels of EBV in mononuclear cells or in plasma by PCR is the key to an early detection of PTLD. Serial EBV quantitative PCR levels are the most important monitor as rising levels are more predictive than a single positive EBV quantitative PCR. EBV+ PTLD can arise at any time after transplantation, but the incidence is highest in the first 1–2 years post-transplant, so monitoring should be frequent during this period [39]. Unfortunately, there is currently no standardized assay for EBV quantitation and multiple PCR-based platforms have been used. Thresholds for elevation of the EBV load are therefore established by individual labs [39]. A negative EBV PCR does not exclude the possibility of PTLD. Unexplained anemia, thrombocytopenia or leukopenia, and high LDH and uric acid levels are non-specific generic abnormalities, associated with PTLD or another malignant blood or lymph disease. Patients should be carefully checked for tonsillar hypertrophy; if they do occur, a biopsy is indicated. Radiological monitoring includes MRI, CT, and positive positron emission tomography (PET) scanning to evaluate the spread of PTLD. In case of CNS involvement, a lumbar puncture with cerebral spinal fluid (CSF) analysis (EBV PCR in CSF fluid) is indicated. Histopathologic examination of the tumor can only determine the definitive diagnosis.

A new development in the monitoring process is the detection of a certain viral load as proxy for decrease of immuno-competence [40]. Analyses in 96 heart and lung transplant recipients suggest that high levels of anellovirus load may be indicative of over-immunosuppression. In line with this, metagenomic shotgun sequencing of tissues from PTLD patients showed that more than 50% of the specimens contained anellovirus sequences, and the anellovirus levels, but not EBV levels, were associated with death within 5 years in a univariate analysis [41]. This presumes that virome changes, and even specific viruses such as anellovirus, may provide indirect measures of the immune status, which can promote the development of EBV+ PTLD.

Therapy/prevention of PTLD. The first step in the prevention of PTLD is reduction of immunosuppressive therapy in case of an increase in EBV load in EBV-seronegative recipients and incase of EBV disease in all recipients (KDIGO level 2D and 1C) [24]. Rituximab is the cornerstone of 1e first-line therapy of PTLD. In a multicenter trial, all 152 transplant recipients with CD20+ PTLD, unresponsive to immunosuppression reduction, were first treated with four doses of weekly rituximab. In case of complete remission, treatment with rituximab was continued with another four doses of rituximab, other patients received four R-CHOP courses. The overall response and CR rates were 88% and 70%, respectively; the response to the first four courses of rituximab was the most significant prognostic factor with respect to overall survival and time to progression [42].

Post-Transplant Diabetes Mellitus

Hyperglycemia and post-transplant diabetes mellitus (PTDM) are serious complications of kidney transplantation [43]. Hyperglycemia is very common in the early post-transplant period, with approximately 90% of adult kidney recipients exhibiting hyperglycemia in the first weeks after transplant. The development of PTDM is associated with reduced graft function, increased cardiovascular disease, higher rates of rehospitalization, and lower patient survival among adult recipients [44, 45].

PTDM is highly prevalent in adult kidney transplant recipients [46], but is less common in transplanted children according to a retrospective analysis of the NAPRTCS registry. The reported incidence of PTDM was reported at 2.6% (2). More recent retrospective studies reported higher incidences of 8–13% [47, 48]. The American Diabetes Association (ADA) criterion has defined criteria for PTDM [43]. Risk factors for PTDM include traditional general diabetes risk along with transplant-associated risk factors that include immunosuppressive medications.

The prevalence of obesity in children is rising and remains a concern among health care providers as it is a recognized cardiovascular risk factor. Nearly one in five school-aged children in the United States has obesity and this may contribute to PTDM [49]. Bondi described a cumulative incidence of obesity at 5 years post-transplant in nearly 25% of all transplant recipients, with pediatric kidney transplant recipients having the highest risk with a 3.13 adjusted hazards ratio [50]. The risk factors for obesity appear to be young age, overweight at the time of transplant, race, and relative high cumulative steroids use [50, 51].

Additionally, glucocorticoids, mTORi, and CNI are known risk factors, and support's individualized immunosuppression regimens in select patient populations [51]. Glycemic effects of glucocorticoids are thought to be dose and duration dependent, with higher doses of glucocorticoids in the first 6 months being a particular risk factor based on NAPRTCS data [52]. CNI are directly toxic to the islet cells and impair insulin gene expression and insulin secretion [51]. Studies have demonstrated tacrolimus and sirolimus as independent risk factors for PTDM. Changing to cyclosporine or belatacept has been shown to improve or resolve diabetes without graft compromise in adults [51, 53]. The effects are dose related and may be partially reversible [54, 55].

The gold standard test for the diagnosis of PTDM is the oral glucose tolerance test, but screening hemoglobin A1c and fasting blood glucose values can identify high risk patients. The diagnosis of PTDM is optimally made when patients are on a stable immunosuppressive regimen and without infection [43]. Treatment of PTDM should follow the ADA guidelines with overall multifactorial management that includes lifestyle modification, alternative immunosuppressive regimens, and pharmacologic intervention [43]. Insulin is often the first-line therapy as oral antihyperglycemic agents have limited safety and efficacy data in pediatric kidney transplant recipients with PTDM [56].

Bone Disease Following Transplantation and Growth

Bone Health

Mineral bone disease after kidney transplantation is a spectrum of bone abnormalities related to CKD and the use of corticosteroids. The process starts early in CKD and assessing bone health is a key element of managing kidney transplant recipients; CKD-related bone disease is described at length in ▶ Chap. 72, "Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD)". Post-transplant mineral bone disease is often due to preexisting CKD-MBD, glucocorticoids, and reduced allograft function [57]. Dysregulation of the pathways responsible for bone health can lead to bone demineralization, short stature, pain, deformities, and anomalous calcifications [58].

Biochemical anomalies can be seen in calcium, phosphate, parathyroid hormone (PTH), 25-hydroxy vitamin D, 1,25-dihydroxyvitamin D, alkaline phosphatase, and Fibroblast Growth Factor (FGF) 23 in pediatric kidney transplant recipients [58]. Analysis of the European Society for Paediatric Nephrology/European Renal Association-European

Dialysis and Transplant Association Registry demonstrated many abnormalities in pediatric kidney transplant recipients. Abnormal serum phosphorus levels were seen in 25%, serum calcium in 30%, and hyperparathyroidism in 41% of the recipients [59]. Persistent elevations in PTH and FGF23 may cause hypophosphatemia and resulting impaired bone mineralization [57]. The risk for fracture and impact on bone density has not been widely studied in pediatric patients and data is limited [60].

Additional risks for bone mineral disease appear to be related to racial differences among pediatric kidney transplant recipients related to bone disease. Black race was associated with 20–25% higher PTH values and lower bone density in chronic kidney disease and kidney transplant recipients [61, 62]. Hispanic race was associated with a 17.5% higher PTH and 16% lower C terminal FGF323 values compared to white chronic kidney disease and dialysis patients [62, 63]. The studies in pediatric kidney transplant recipients and racial differences have been limited and more data is needed.

Growth

Growth failure is a commonly observed complication of ESKD largely due to deficiency, resulting in pediatric kidney transplant recipients needing to grow. The NAPRTCS registry has estimated the height deficit at the time of transplant to be −1.8 [64], which is in line with European data. The greatest risk factors are relative older age at time of transplant, allograft dysfunction, and corticosteroid use [65–67]. Younger children, less than 5 years of age, can demonstrate catch-up growth, but older children rarely demonstrate catch-up growth [66].

Sustained growth failure remains common after renal transplantation as a result of glucocorticoid therapy and renal impairment [65]. Steroid avoidance demonstrates particular improvements in height standard deviation score (HSDS) in the first year after steroid withdrawal and in prepubertal patients [67]. Reducing or avoiding corticosteroid therapy can be safe in select kidney transplant recipients and will have beneficial impact on linear growth [67, 68].

The safety of growth hormone therapy had been a concern in pediatric kidney transplant recipients in the 1990s. Recent work has shown that growth hormone is safe and efficacious with an improved HSDS of 0.68 in transplant recipients receiving growth hormone compared to controls [69, 70].

Urologic Complications

Urological complications have significant impact on cost and frequent hospitalizations in the post-transplant period. The incidence was higher in children with a history of cystoplasty and preexisting urologic surgeries/anomalies [71, 72]. With approximately 25% of children undergoing kidney transplantation having anatomic abnormalities of the lower urinary tract, urologic complications remain a major concern [73].

Ureteral Obstruction

Risk factors for ureteral obstruction are not well understood, but contributing factors include surgical expertise, decreased vascularity of the donor ureter, and rejection [74]. Early ureteric obstruction is usually related to potential surgical complications, such as lymphocele or hematoma. The frequency of surgical complications has reduced from 30% to around 10% with improvements in donor extraction, preservation of vasculature, and use of a shorter ureter [75, 76].

Causes of obstructions that occur after 1-month post-transplant are more elusive. Adult literature suggests that obstruction at the uretero-vesicular junction is most susceptible to ischemic injury as the vascular supply is derived from the donor renal hilum. Mid- and uretero-pelvic junction obstruction is more commonly related to rejection with localized ischemic injury [72].

BK virus (BKV) is a common polyoma virus with seropositivity in 60–90% of the general adult population [77, 78]. In kidney transplant recipients, BKV can reactivate and cause viruria, nephropathy, ureteral ulceration and stenosis, interstitial nephritis, and, rarely, hemorrhagic cystitis [78, 79]. The risk factors for ureteric stenosis and BK reactivation are thought to include active

BK infection in the donor, ischemic injury, immune control, and delayed graft function [78, 80].

Dysfunctional Lower Urinary Tract

Transplantation using a dysfunctional bladder is associated with many urologic complications. These urologic complications may negatively affect graft survival, although results are mixed in the literature [81–86]. Careful surveillance through urodynamics and urologic care are likely needed to optimize these outcomes.

Success with bladder augmentation and ileal conduit diversion have demonstrated good short- and medium-term results, despite non-standard drainage, in more severe cases of lower urinary tract dysfunction [87–89]. If no intervention is conducted for severe bladder dysfunction, clean intermittent catheterization has been shown to be safe and effective [90]. Urinary tract infections and ureteral complications remain one of the leading complications when transplanting into a dysfunctional lower urinary tract [87, 91].

Urinary Tract Infections

Urinary tract infections (UTI) are the most common bacterial infection in kidney transplant recipients in both adults and children and are the leading cause of hospitalization in children [92–94]. Although UTIs can occur anytime in the postoperative period, it is most likely to occur in the first 3–6 months [95]. Transplant-specific risk factors include the presence of urethral stent, vesicoureteral reflux (VUR), episodes of acute cellular rejection, receipt of a deceased donor transplant, duration of hemodialysis, history of UTI prior to transplantation, and potentially antithymocyte globulin induction [95].

Early febrile UTIs, within the first 6 months of transplant, are associated with higher rates of graft loss and patient death based on data from the United States Renal Data System (USRDS) [96]. Patients with lower urinary tract dysfunction, females, and patients with urologic disease as their cause of kidney failure are at particularly higher risk of UTIs [84, 97]. Most kidney transplant recipients receive trimethoprim/sulfamethoxazole (TMP/SMX) as primary antimicrobial prophylaxis for *Pneumocystis jirovecii* pneumonia (PJP) for the first 6–12 months. TMP/SMX is also effective at preventing UTIs, but is usually used at higher doses than traditional PJP prophylaxis. The American Society of Transplantation Infectious Disease Community of Practice recommends utilizing doses for PJP prophylaxis as opposed to UTI prophylaxis given the risks for renal dysfunction and hyperkalemia with TMP/SMX. For patients who cannot tolerate TMP/SMX, 1 month of nitrofurantoin prophylaxis or cephalosporins is recommended [95].

The approach to recurrent urinary tract infections involves assessment of the underlying etiology and targeting prevention. Evaluation should include assessment of post-void residual bladder volumes and ultrasonography of the kidney and bladder to assess for structural anomalies. If no anomalies are present, a voiding cysto-urethrogram is recommended [95]. Patients should be reminded of basic infection prevention measures that include hydration, regular voiding, wiping front to back in females, avoiding bubble baths, and voiding after sexual intercourse.

Depending on the transplant surgeon, the use of anti-reflux surgery technique may or may not be done at the time of ureteral anastomosis for fear of creating a secondary renal obstruction. In fact, the adult literature supports a high rate of vesicoureteral reflux (VUR) post-transplant at 41% [98].

The incidence of VUR in pediatric kidney transplant recipients is not well studied, but seems to be higher than in adults. Single center studies found an incidence of symptomatic VUR to be between 4% and 12% and patients with underlying bladder pathology were at higher risk for developing VUR [73, 91, 99].

Surgical revision of the vesicoureteral anastomosis can be challenging due to scar tissue at the bladder and the tenuous vasculature of the transplanted ureter. Surgical intervention should be reserved for those who are having recurrent, febrile UTIs [99, 100]. Afebrile UTIs associated with vesicoureteral reflux can be managed with bladder training, hygiene, and/or antimicrobial prophylaxis [100]. Lengthening the submucosal tunnel and ureteral reimplantation have been shown to effectively improve or resolve VUR in pediatric kidney transplant recipients, but the impact on GFR decline may not be as impactful as initially thought [91, 101].

Gastrointestinal Complications

Gastrointestinal pain, diarrhea, and vomiting induced by mycophenolate mofetil is one of the most common early complications after transplantation. Spreading the dosage to three times per day or switching to enteric-coated mycophenolate can be effective in reducing these side effects with equal immunosuppressive efficacy [102]. Patients treated with tacrolimus are significantly more likely than those receiving cyclosporine to suffer dyspepsia, vomiting, diarrhea, and hypomagnesemia [103].

Cosmetic Complications

Cosmetic side effects of immunosuppressive therapy are among the most underrated complications of solid organ transplantation in children, especially in adolescents. Hirsutism and gingival hypertrophy induced by cyclosporine (and further enhanced by calcium channel blockers) in combination with the cushingoid side effects of steroids have often induced serious depression in many adolescents and caused a significant nonadherence to the therapy with subsequent graft loss [104]. The recent steroid-free, tacrolimus-based regimens have largely dealt with this problem, although tacrolimus-induced alopecia can also be a serious side effect.

Thrombotic Microangiopathy

De novo thrombotic microangiopathy (TMA) is a rare complication following transplantation. Reported rates are from 1.1% to 14% in adults. Allograft biopsy is always the gold standard of diagnosis. Pathology defines TMA as glomerular, arteriolar, or interlobular artery lesions characterized by patchy distribution with intimal cell proliferation, thickening, and necrosis of the wall, thrombi, and luminal narrowing [105].

TMA has heterogeneous manifestations in the post-transplant setting and is a complex and challenging process. De novo TMA may appear localized or systemic; localized TMA is only detected by biopsy and may lead to slowly chronic deterioration of graft function whereas systemic TMA may lead in the early postoperative period to rapid graft loss [106]. Even in systemic TMA, these systemic sequelae are relatively mild compared to non-transplant TMA and include features of hemolytic anemia, thrombocytopenia, and schistocytes.

De novo TMA may be secondary to calcineurin inhibitor treatment, mTORi, and infections, and antibody-mediated rejection [107–109]. ABO-incompatible kidney transplantation is an independent risk factor even after desensitization [106]. There are limited studies in pediatric kidney transplant recipients regarding TMA. In case reports of de novo TMA from the adult and pediatric literature, TMA was successfully treated with conversion from calcineurin inhibitors to alternative immunosuppression [110–112].

Neurological Complications

Neurological complications are common in adults after solid organ transplantation with a reported prevalence varying from 10% to 59% [113]. The reports in children are scarce and the prevalence seems to be lower than in adults, but far from absent. Neurotoxicity of immunosuppressive drugs, especially of calcineurin inhibitors accounts for most neurological complications. Clinical features may vary from tremors (up to 55% of patients), paresthesia, and seizures up to severe conditions like disabling pain syndrome and posterior reversible encephalopathy syndrome (PRES). Calcineurin-related PRES in older literature is often described as leukoencephalopathy and has also been reported in children [114, 115]. It presents as rapid onset of confusion, decreased level of consciousness, severe headaches, hyperreflexia, visual changes or seizures, and typical posterior white matter edema on imaging. Dysregulation of the cerebral perfusion and endothelial dysfunction have been linked to PRES. CNS toxicity is not related to plasma levels of cyclosporine or tacrolimus levels and children younger than 6 years of age seem more vulnerable for cyclosporine-associated neuropathy [116]. Tacrolimus seems to induce even more neurotoxicity than cyclosporine in adults [103], but data in children is lacking. Peripheral neuropathy is often seen in adults but may also occur in children [117]. It is most prevalent in chronic dialysis patients, but it may persist after transplantation. A neurophysiological study on 31 kidney transplanted children and adolescents showed that 19% of patients had neuropathic changes and that graft dysfunction and anemia were associated factors [118].

Quality of Life

Kidney transplantation increases life expectancy and much research has been conducted on medical outcomes in pediatric kidney transplantation. It has been presumed that the improved life expectancy improves quality of life (QOL) for children with ESKD, but the sequelae of transplantation and its treatment can make it difficult for transplant recipients to have similar quality of life to their peers. Data on health-related quality of life (HRQOL) of kidney transplanted children are conflicting. In a Dutch-Belgian-German study on 42 dialysis and 150 transplanted children, both dialysis and transplanted children scored significantly lower scores on all domains, except for emotional in the preemptively transplanted group. Preemptively transplanted patients scored higher on physical domains, but not on other mental domains. School functioning was equally low in dialysis and transplanted patients, even in preemptively transplanted patients [119]. A study in 26 Australian kidney transplanted adolescents reported overall good HRQOL, but with remarkable decrements in cognitive functioning and emotion [120]. Others confirm that preadolescents seem to report low HRQOL than adolescents after transplantation. Other important risk factors are neurological comorbidity, shorter follow-up time, and family-related variables. The latter stresses the potential need for family interventions in certain patients [121]. Like many things, QOL is subjective and some transplant recipients did not feel transplantation provided the picture of wellness they were expecting [122]. The conclusion is that restoring an organ function does not necessarily restore a “normal life” in children. Young adults who have experienced ESKD, even those who underwent kidney transplantation, were noted to have a variety of perceived challenges. These include a desire for normality, disrupted or modified ambitions, and a sense of uncertainty [123]. Despite the health benefits of transplantation, ESKD seriously affects QOL of children, even after transplantation.

Peer and Family

Compared to their peers, pediatric kidney transplant recipients are three times as likely to live in the family home compared to the general population and less likely to be married or live with a partner [122, 123]. Young adults are reluctant to share their medical condition with peers for fear of rejection or being considered “abnormal” [122, 124]. Many express body image issues related to their scars and medication side-effects (e.g., weight gain and hirsutism). They feel shame over being different and develop a degree of social isolation [123].

Education and Employment

Adults with ESKD have delayed education compared to peers, negatively impacting education [124–126]. Children under 12 years of age when diagnosed with ESKD had more difficult educational achievements compared to those who developed ESKD after puberty [124]. Children with ESKD were less likely to complete high school or higher education compared to peers [126–128]. Cultural differences may also impact QOL in school functioning, with non-Western origin transplant recipients in one study having higher risk of impaired school functioning compared to Western countries [129]. Additionally, young adults transplanted as children are less likely to have a job compared to the general population and adults who developed ESKD [122–126].

Cognitive Functioning

Cognitive impairment has for long been recognized as an important comorbidity of severe CKD and has particularly been linked to chronic dialysis treatment. The Neurocognitive Assessment and Magnetic Resonance Imaging Analysis of Children and Young Adults with Chronic Kidney Disease (NiCK) workgroup found significantly poorer performance of cognitive performance, particularly with respect to attention, memory, and inhibitory control among patients with CKD aged 8–25 years, compared to healthy controls [130]. The extent of decrease in eGFR, and increased diastolic BP load were independent predictors for low performance. There is evidence for anatomic substrate for functional brain impairment as has been assessed in patients with severe kidney dysfunction. White and gray matter brain atrophy assessed by CT and nonquantitative MRI has previously been described in young CKD patients and in particular in dialysis patients [131, 132]. The NiCK study has reported smaller brain volumes in transplanted patients, but no abnormalities in pre-dialysis patients aged 8–25 years. In this study, there was no association found with extent of eGFR loss, but transplanted patients had the most prominent loss of brain volumes as compared to conservatively treated patients with CKD. Separate comparisons of non-transplant and transplant CKD patients revealed only significant differences between the transplanted group and controls with respect to brain volumes. Unfortunately, no dialysis patients were included in this study [131]. In Belgium and the Netherlands, we performed a comparable study among 24 patients with CKD <30 aged 8–30 years (7 conservatively treated, 7 on dialysis, 10 transplanted for at least 2 years). We found smaller white matter volume and smaller volumes of subcortical structures (i.e., limbic system and basal ganglia) in all dialysis and transplanted patients, but not in conservatively treated patients. Dialysis and transplanted patients had equally smaller gray matter and subcortical volumes than healthy controls. Longer dialysis duration was predictive of smaller gray matter volume. DTI analysis indicated that dialysis and transplanted patients had lower white matter integrity in a widespread cluster of primarily distal white matter tracts compared to healthy controls [133]. The MRI findings were significantly associated with cognitive performance dysfunction (Lijdsman et al., in process). We found no difference in brain abnormalities between preemptively and non-preemptively transplanted patients, although the numbers were too low for a reliable comparison. We also found that a longer time post-transplantation during the patients' lifetime was related to worse white matter integrity. This could imply that CKD-induced brain abnormalities may not be reversible after a successful renal transplantation and may even worsen over time, potentially reflecting that CKD-induced brain damage become more pronounced while brain development continues (e.g., "growing into deficits") or it reflects delayed axonal degeneration (e.g., "Wallerian degeneration"). The fact that dialysis and transplanted patients – even preemptively transplanted – had comparable brain abnormalities suggests that not uremia itself, but rather microvascular damage or direct neurotoxicity by immune-suppressive maintenance therapy may play an important role in the pathophysiology of CKD-induced brain damage [134]. HTN and impaired cerebral perfusion is indeed another often mentioned potential determinant in CKD [135, 136]. This hypothesis fits with the specific localization of structural abnormalities revealed in the Dutch-Belgian study, as widespread disruption of white matter integrity has been previously been described in adult patients with HTN [137], which is a risk factor for cerebral perfusion. This speculation is in line with a recent review, concluding that CKD-related HTN may be a far more important risk factor for the influence of pediatric CKD on the brain than initially thought [135].

Impaired cognitive function may have a severe impact on the QOL of transplanted children and adolescents, as was shown in an Australian study on health-related QOL in adolescent kidney transplant recipients. Although overall scores were high, there were also important decrements in specific QOL domains, especially concerning emotion and cognitive dysfunctioning [120].

Late Outcome of Pediatric Transplantation

Data on long-term outcome of kidney transplantation should be interpreted in the context that they concern outcomes of patients that have (partly) been treated with currently outdated therapeutic regimens. A shortcoming of the existing data is that they are most often based on large and incomplete registry studies. Finally, the effects of long-term transplantation are to a certain extent often confounded by the carry-over effects of other types of kidney replacement therapy (KRT), i.e., hemodialysis or peritoneal dialysis, as the majority of patients have undergone different modes of KRT.

One of the few comprehensive cohort studies on late somatic and psychosocial outcome was a relatively small study on all 249 Dutch patients aged <15 years at onset of chronic renal replacement therapy between 1972 and 1992 and who were born before 1979, performed in 2000 and 2010, the so-called LERIC study. Thanks to the reliable registry system in the Netherlands, all patients who fitted the inclusion criteria could be traced. As nearly 80% of the time on KRT had been with a functioning graft in the late survivors, most outcomes reflect at least to a great deal the burden of transplantation. We will discuss outcomes in relation to other existing data on late outcome.

Late Graft Survival and Number of Transplants Over Time

Despite the potential increasing hazard of graft loss due to immunization, long-term follow-up data show that patients indeed can have multiple transplantations and that even the third or fourth transplantation in a patient can have a long survival. In the LERIC study, patients had changed their KRT modality between 1 and 11 times after a median KRT time of 25.5 years in 2010 [138]. The number of transplantations had been 1 in nearly 40%, 2 in 36.4%, 3 in 18.6%, 4 in 3.5%, 5 in 0.4%, and 6 in 1.3% of the 152 survivors by 2010 and after a median total KRT time of 25.5 years. Of all transplanted patients, 31% lived for more than 20 up to 37.2 consecutive years with a single graft. In some cases this was the third or fourth transplantation [139]. In 2000 and 2010, 75% and 79% of the surviving patients lived with a functioning graft. The ages of survivors in 2000 and 2010 ranged from 20–40 to 30–50 years, respectively. In a study on late outcome of 300 transplanted patients between 1973 and 2000 at Great Ormond Street Hospital in London, with a follow-up of 2.5–29 years, the median graft survival was 10.8 years (95% CI 6.9–14.6) and 46% and 37.9% after 15 and 20 years [140].

Late Overall and Cardiovascular Mortality

Both ANZDATA and LERIC studies on long-term survival in patients aged <20 years with KRT onset 1962–1992 found a 30 times increased mortality after a mean of 15 and 12 years of KRT time, respectively [141, 142]. The Great Ormond late outcome study on transplanted children with a median follow-up of nearly 20 years mentioned 37 deaths on 300 transplanted patients, a significant lower figure, reflecting the relative favorite situation of transplantation over dialysis with respect to survival [140]. Cardiovascular disease was most often reported as cause of death in both Australian and Dutch studies and in a German long-term follow-up study (41%, 43%, and 50% of cases, respectively [141, 142], but also in the Great Ormond transplantation long-term outcome study (32%) [140]. A long-time burden of HTN, onset of KRT at <6 years of age, onset of KRT before 1982 and, most important, the cumulative dialysis duration were significantly associated with premature death in the LERIC study [141]. Ten years follow-up of the LERIC study (25 years of mean KRT time) showed a significant trend from cardiovascular disease to infections and malignancies over time as the most important cause of death, reflecting the increasing impact of transplantation over time. However, an increased awareness over time among physicians of CVD as important threat and an overall change in lifestyle might also have influenced the reduced prevalence of CVD-related death. In 2000, uncontrolled HTN had been highly prevalent among the transplanted survivors, whereas in 2010, survivors had on significant lower BP, used more antihypertensive drugs – especially more ACE-inhibitors – had had more often cholesterol lowering medication and less often left ventricular hypertrophy on cardiac ultrasound [138].

The late outcome data may overestimate the mortality risk of the current pediatric KRT population, as preemptively transplantation has become the first choice of therapy in countries which has reduced the total time on dialysis in pediatric onset ESKD patients over time. Registry data show indeed improved outcomes of childhood ESKD over 40 years of KRT, but at the same time a dramatic slowdown of this improvement over the last 25 years [141–145]. The ANZDATA, LERIC, and a Canadian long-term outcome study reported no increase in survival after 1983 or 1992 (Canada), respectively [138, 142, 143]. In line with this, the adjusted 10 years survival rates of patients with onset of KRT at age 0–21 years according to the USRDS in 1996 and 2003 were equal (71.3% and 71.8%, respectively) [145]. This development may be partly due to the fact currently more infants and disabled patients are accepted for KRT than in the 1980s and 1990s of the last century, but the fact remains that the mortality rate has remained unacceptably high. Other studies have confirmed the impact of CVD on premature death in patients with childhood-onset KRT [1, 139, 142–144, 146].

Both the ANZDATA and Canadian Registry studies show similar results with respect to dialysis and young age as risk factors, but in both studies the increase in mortality risk due to dialysis only became significant in patients with a dialysis vintage >2 years [142, 143].

Other Comorbidity

In 2000, >40% of survivors in the LERIC study (median age 30 years), 75% of them with a functioning graft, suffered from comorbidities that affected their activities of daily life. This concerned chronic fatigue, symptoms related to CVD (shortness of breath, recurrent angina), symptoms related to peripheral neuropathy, or disabilities due to locomotor disorders [147–150]. In 2010, more patients reported more often chronic fatigue as important, affecting daily life and negatively impacting the ability to participate in the labor market [151].

Cardiovascular Disease

In 2000 we assessed in 140 of 187 surviving patients for surrogate markers for CVD. Nearly 50% of all living male and 40% of all female LERIC patients were found to have moderate to severe left ventricular hypertrophy (LVH), 75% of them being transplanted for >2 years at the time of the investigation [148]. Arterial wall stiffening was significantly more prevalent in all patients than in age-matched peers [149]. In contrast, between 2000 and 2010 only 1 patient out of 34 died of CVD [138]. Among the survivors, the prevalence of cardiovascular risk factors decreased from 41.3% in 2000 to 18.8% in 2010. The odds ratios (Ors) in 2010 relative to 2000 for LVH, HTN, and hypercholesterolemia were 0.26, 0.22, and 0.04, respectively. ACEi and cholesterol-lowering medications were prescribed significantly more often in the period 2000–2010 than in previous years (OR 7.4 and 11.5, respectively) [152].

These outcomes reflect the burden of CVD in young patients with pediatric ESKD, but also the reversibility of CVD during the years of transplantation, provided strict and effective cardioprotective management is followed. Studies on CVD in children with ESKD confirm that LVH may occur early in life, is strongly associated with HTN, and may be reversible after transplantation as long as HTN is well controlled [4, 153]. This underscores the importance of BP control in both young transplanted and dialyzed patients. There still seems much to be learnt in this area, as relatively recent data of the European Society for Pediatric Nephrology/European Renal Association–European Dialysis and Transplant Association registry and North American Pediatric Renal Trials and Collaborative Studies mention 40–65% of children on KRT with uncontrolled HTN [4, 5, 154, 155]. A Belgian-Dutch study from 2016 in transplanted patients 8–30 years of age showed a more favorable picture during childhood, but also a sharply increased percentage of uncontrolled HTN in the age group 25–30 years [8].

Infections

In the LERIC population, infection has become the most important life-threatening comorbidity over time [138]. Between 2000 and 2010, 44% of 34 casualties died of infections. The contribution of infection to hospitalization increased significantly during the last decade in transplanted patients, with an increase in the hospital admission incidence rate ratio for infections over other causes from 0.25 to 1.0. The admission rate for urinary tract infections (UTIs) also significantly increased over decades in transplanted patients [156].

In adults, infections are the leading cause of death in the first year after kidney transplantation [157]. UTI (69.8%) and CMV infection are most frequent during the first 6 months, but UTI is also highly prevalent much later after transplantation. In a retrospective cohort study on 28,942 Medicare renal transplant recipients, the cumulative incidence of UTI was 60% for women and 47% for men at 3 years after transplantation. Late UTI was significantly associated with an increased risk of subsequent death [adjusted hazard ratio IAHR) 2.93] and graft loss (AHR 1.85) [158].

Data from adult studies suggest a relationship between an increase in the intensity of immunosuppressive maintenance therapy and an increasing incidence of serious infections. This association seems related to the increase in dose rather than to the use of a different type of potentially stronger immunosuppression [159, 160]. LERIC patients have in general been treated with relatively mild immunosuppressive regimens when compared with most current strategies in transplanted children. This makes the findings in the LERIC patients particularly alarming for the current group of young transplant recipients. Lowering the immunosuppressive load, especially in patients with a prolonged rejection-free period after transplantation, might reduce the number of fatal infections.

Malignancies

Based on several reports on malignancies after pediatric solid organ transplantation, the cumulative incidence of cancer seems to increase from 7%, by 10 years to 13–15% by 20 years and 26–41% by 30 years post-transplantation [32]. The incidence varies among studies due to differences in patient characteristics, type of transplantation, geographical (UV) influence, and cancer screening practices. The long-term risk of cancer among pediatric solid organ transplantation is 12–33 times greater than the general population [32]. In 2010, 54 (21%) LERIC patients of the Dutch cohort study had developed 105 de novo malignancies after a median KRT time of 25.5 years. The incidence rate and incidence rate ratio for cancer was 2.0 and 21.7, respectively, for all patients and 26.0 and 81.5, respectively, for patients 45–50 years of age. The cancer-free survival after 20, 30, and 35 years of follow-up was 87%, 68%, and 58%, respectively. Of all transplanted patients, 30% had died without malignancy, 33% had at least one malignancy, and only 36% was alive without malignancy in 2010. A cumulative incidence competing risk analysis showed that after 30 years of transplantation of all survivors, 41% had developed cancer and 31% had a second de novo cancer within 1 year after the initial cancer diagnosis. The median age at malignancy diagnosis was 33.5 years (range 11.0–49.0) and the median KRT time was 25.0 years. Death was attributed to a malignancy in 13 (24%) patients [161, 162].

Skin cancer was by far the most common type of cancer in the LERIC study, even more pronounced than in other studies [161–164]. The adjusted risk for non-melanoma skin cancer (NMSC) was increased >200 times and the majority occurred at a later age than previously reported, i.e., 39.1 years (range 21.8–50.4). Including metastases, 114 NMSCs [78 squamous cell carcinoma (SCC) and 36 basal cell carcinoma (BCC)] occurred in 39 patients. The overall incidence rate ratio for SCC was 744 and for patients 45–50 years of age it was 2610. Two patients died of SCC; far more patients had important morbidity because of SCC metastases. There were five melanomas occurring at a median age of 31.1 years, seven episodes of post-transplant lymphoproliferative disease (PTLD) occurred in six patients at 0.6–20.8 years after the first transplantation and nine solid non-skin/non-lymphoproliferative tumors, all of different origin, at a mean age of 41.7 years, consistent with a rate of 158/100,000 patient-years [162].

Other pediatric ESKD studies have found a lower malignancy incidence than that in the 2010 LERIC extension, but in line with the LERIC data of 2000, which reflects the relatively late onset of cancer after transplantation [146, 165–167]. The predominance of SCC over BCC seems to be more pronounced in pediatric than in adult transplant recipients (2.8:1 versus 1.7:1) [163]. SCC is primarily found in parts of the body that are exposed to sunlight, may develop rapidly, and recurrences are common [168] (Fig. 1).There is evidence that ultraviolet light exposure and human papilloma virus (HPV) in combination with immunosuppression – especially azathioprine – are potential causative factors for SCC [169, 170]. Switching from tacrolimus or cyclosporine to sirolimus has been shown to be preventive against the development of new SCC among kidney-transplant recipients with previous SSCs [171]. SCC may appear in the absence of any preexisting skin lesion, but is often preceded by actinic keratosis, which suggests HPV involvement in its pathogenesis (Fig. 2). A recent study showed in a skin cancer model how musculus papillomavirus 1 infection caused cSCC in cyclosporine-treated mice, even in the absence of UV light [172]. Recently, it has also been shown that in a mice study how vaccination against HPV may protect against skin cancer development. In this study, mice with natural or acquired immunity against mouse papillomavirus type 1 (MmuPV1) were protected against skin carcinogenesis induced by ultraviolet radiation or chemicals in a manner dependent on CD8+ T cells [173]. Lastly, it has been shown that polygenic risk scores (PRSs) calculated from genome-wide association studies of NMSC in a general, non-transplant setting, also predict risk of and time to post-renal transplant skin cancer [174].

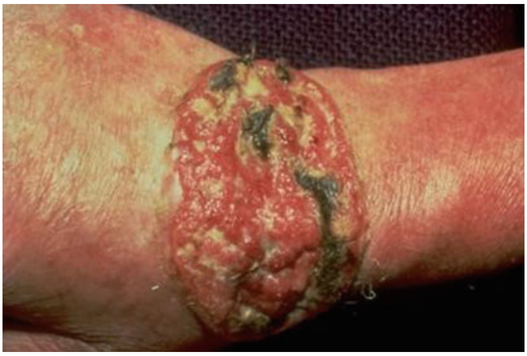

Fig. 1 A 42-year-old man with recurrent squamous cell carcinoma 31 years after pediatric kidney transplantation (with permission from patient)

Both LERIC and the Israel Penn Transplant Tumor Registry Study data show an earlier onset of melanoma compared with NMSC [162, 163]. Melanocytic nevi, a risk factor for melanoma, may develop in excess after transplantation [175]. In transplantation, melanoma can be transmitted by the donor. Persons with a history of melanoma should be excluded from donation. Sun protection and removal of suspect nevi are the most important protective measures.

PTLD has biphasic onset with a first peak at 6–12 months and a second 5–10 years after transplantation. Early-onset PTLD is associated with active EBV infection in 95% of patients and has a better prognosis than the late-onset form [33, 176]. The incidence of PTLD increased up to the early 2000s, but more recent United Network for Organ Sharing data have shown a downward trend among adult kidney recipients who were transplanted between 2007 and 2009 as compared to those transplanted between 2000 and 2003, all due to a decrease in EBV-associated PTLD [176]. Early detection of EBV activation by polymerase chain reaction and subsequent adjustment of immunosuppression may have positively influenced this trend.

Renal cell carcinoma is the most common solid non-skin carcinoma after transplantation and often affects the native kidneys. The fact that the incidence is higher than in HIV patients may indicate involvement of local renal factors, more than immune suppression as risk factor [177].

Bone Disease and Motor Disabilities

Over two-third of the LERIC population was severely growth retarded (height <−2.5 SD), as expected in patients from the "pre-growth hormone era." More than one-third daily suffered from bone pain or had disabilities related to metabolic bone disease. About 18% were severely disabled because of bone disease [147]. Uncontrolled chronic kidney disease-mineral and bone disorders (CKD-MBD) during growth in addition to transplantation-associated factors, such as glucocorticoid-induced suppression of bone formation, calcineurin inhibitors

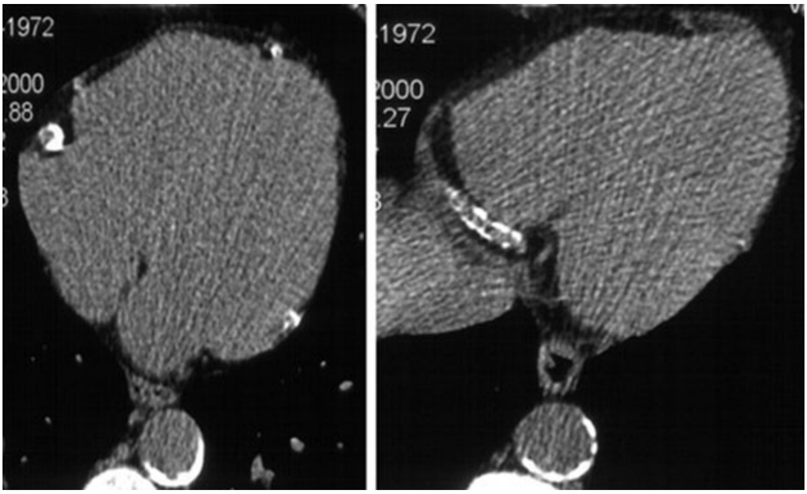

Fig. 2 CT sections from a 27-year-old male on hemodialysis with childhood-onset of ESKD showing extensive calcification in all three coronary arteries and aorta. (From Oh et al. [139])

(CNIs) and persistent hyperparathyroidism may all have added to this situation [147, 167]. The use of bisphosphonates has been shown to improve bone mineral density in transplanted patients, but the positive effect on fracture risk and clinical improvement of BMD has still to be demonstrated [178].

Cognitive Functioning

In 2000, we found intelligence quotient (IQ) scores among the LERIC patients to be on average 10 points lower than in the age-matched Dutch population, in line with studies on IQ performed in CKD children [125, 179]. The most important determinant of impaired schooling and cognition was a relatively long period of dialysis during youth, but we found no differences in intellectual performance between patients on dialysis or transplanted patients at time of assessment. Most deficits were found in tasks requiring concentration, memory, and general knowledge [125]. These deficits are reflected in cognitive impairment in adult hood [125, 180]. Data on the reversibility of cognitive impairment after transplantation are conflicting, and may be different in children than in adults [133, 181–183].

Quality of Life

Both mental and physical health-related QOL scores of transplanted LERIC patients were similar to those of the age-related general Dutch population in 2000, but physical scores were significantly more often impaired in 2010 than in 2000 [184, 185]. Although partly explained by a normal aging effect, the LERIC transplanted patients appeared to be “early agers” in this respect, and the ongoing deterioration, over and above relatively low scores in some domains, could become a significant problem for these patients in the near future. A transplanted woman, age 42 years, illustrated this through the following comments: “Much more often than 10 years ago, even when I was on dialysis, I feel very tired nowadays, reason why I recently have decided to stop working for a while.” According to the same study, all domains relating to psychosocial functioning had remained stable over the last 10 years [185]. Being employed was associated with a lower risk of impaired QOL in the domains of physical functioning, vitality, and general health perception for all LERIC patients. Having offspring was associated with less impaired social functioning and a relatively high income was associated with fewer role limitations due to physical problems [184, 185].

All, except one Japanese study, emphasizes the improvement in QOL after renal transplantation in young recipients, but these favorable outcomes are reported after at most 10–20 years of follow-up [186–188]. The high mental QOL scores of dialysis LERIC patients sharply contrast with the very poor mental scores found in age-matched dialysis patients with adult-onset ESRD in the Dutch NECOSAD-2 study [184, 189], but are consistent with findings in other QOL studies in adults with chronic childhood diseases, such as sickle cell disease and cystic fibrosis [190]. Different coping strategies and expectations of life and different coping strategies between children and adults may explain this difference in mental status. It fits with the hypothesis that health-related QOL is, to a large extent, based on the difference between health expectations and health experiences. Nearly 80% of the LERIC patients stated that their disease had brought them something positive in life. The perceived benefits of having ESKD included more satisfaction with (small things in) life, having developed a sense of perseverance, and positive responses from friends and relatives [185].

Social Outcomes

Employment. In 2000, 67.4% of the LERIC patients were employed [191]. Involuntary unemployment was reported in 19.1% versus 6.4% in the Dutch population of similar age. Most patients (53%) had low-skilled and only 10% had high-skilled professions, a situation significantly different from the average Dutch population. In 2010, 61.8% were still employed; of these patients, 81.8% had at least 50% full-time equivalent of paid work [151]. Having motor disabilities, chronic fatigue, and being on dialysis were the most important factors for becoming unemployed. Some patients reported that their contract was not renewed as a direct result of disclosure of their dialysis patient status to their employer. Unemployment was related to patients' low subjective health perception independent of the objective physical condition or KRT mode. Between 2000 and 2010, we found a significant trend toward more highly educated occupations and an increase in patients' educational level. The average income, however, was significantly lower compared with the general population in 2010 (no data in 2000); 35.2% of patients had lost their job between 2000 and 2010, in 32.3% because they were fired and in 45.2% for medical reasons. In 12.1%, employers indicated that the disease state of the patient influenced achievement. About 21% of patients felt that their disease had a significant negative influence on their professional achievements and career [151].

Other data confirm the LERIC findings [167, 180, 182, 185, 188, 192]. In a recent French study in 624 patients transplanted in childhood, fewer patients than expected had a high-level degree, more patients were unemployed and mean incomes were much lower than in French society [180]. Interestingly, patients less often had a permanent contract than the average French employee (66.8% versus 81.8%). This might reflect the observed reluctance of employers to a long-term professional commitment with renal patients, as we observed in the LERIC study. Nearly 50% of French transplanted patients indicated they had suffered from discrimination [180].

Partnership and autonomy. In line with the French study, in 2000 we found sequelae of impaired autonomy in the LERIC cohort [180, 191]. Of the 144 patients, 49 (34%) still lived with their parents. The odds of living with parents versus living alone for LERIC patients compared with age-matched Dutch citizens was 3.3 [95% confidence interval (CI) 2.3–4.7], while the odds for living with a partner was 0.3 (95% CI 0.2–0.4) for LERIC patients compared with age-matched Dutch inhabitants. In 2010, the situation in the LERIC cohort had completely changed: 67.4% were married or lived with a partner and 28 (31.5%) had offspring, compared with, respectively, 74.4% ($P > 0.05$) and 64.8% ($P < 0.05$) in the general population [151]. This delay in starting a relationship could reflect a genuine delay in sexual maturity or a late "social maturity" as has been described in patients with a chronic illness. Patients with disabilities and patients from southern more often than from northern European countries tend to live with their parents. Men more often have problems with finding a life partner than women [151, 180, 182, 188, 192].

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Ethical Issues in Pediatric Nephrology

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Aaron Wightman and Michael Freeman

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Abstract

A diagnosis of kidney disease impacts a child, their family, and potentially their community. While sometimes acute, kidney disease is often chronic with lasting effects and requiring significant treatments. The complexity and chronicity of pediatric kidney disease raises ethical challenges for pediatric nephrologists, children, and their families. In this chapter, we will provide an overview of the principles of biomedical ethics and the best interest standard and harm principle in medical decision-making for children with kidney disease. We will highlight the unique

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ethical issues that arise among the youngest patients and adolescents nearing adulthood. Finally, we will consider issues of justice within practice of pediatric nephrology.

Keywords

Ethics · Justice · Decision-making · Expense · Best interest · Refusal

Introduction

To consider the ethical issues in pediatric nephrology, it is important to begin with the common framework used in contemporary discussion of medical ethics. Children pose a unique challenge to ethical frameworks initially developed around the experience of an autonomous adult patient. While the predominant framework presumes that the patient is fully autonomous and able to express authentic and sustained values to help guide decisions, these capabilities are generally not attributed to children. Additionally, nearly all pediatric patients are cared for within the context of a family. Parents carry responsibilities for the well-being of their child not shared by family members of an autonomous adult patient. Furthermore, society and, in turn, pediatric nephrologists have additional responsibilities for children. It is important to recognize these distinct aspects of children when considering the ethical issues in pediatric nephrology.

Within this chapter, we will briefly review principlism, the most commonly applied framework in medical ethics [1, 2]. Next, we will review the best interest standard and harm principle in surrogate medical decision-making for children with kidney disease. We will highlight the unique ethical issues that arise among the youngest patients and adolescents nearing adulthood. Finally, we will consider issues of justice within practice of pediatric nephrology.

Overview of Principlism

Medical ethics arose from a rich foundation, incorporating the professional ethics of physicians and other health practitioners throughout history as well the insights of both Western religious and secular ethical traditions. Principlism reflects efforts to identify the common values, or “principles,” which were found in these heterogenous sources. In *Principles of Biomedical Ethics* [2], Beauchamp and Childress identified four common, overarching principles represented in the vast majority of medical ethics: respect for autonomy, beneficence, nonmaleficence, and justice (Table 1). Medical care should adhere to these principles to the greatest extent possible.

Principlism holds each principle is of equal value and importance. Difficult questions in medical ethics typically arise when these principles conflict with each other. We will examine the complex aspects of applying these ethical principles within pediatric nephrology in the remainder of the chapter.

Pediatric Surrogate Decision-Making: The Interface of the Principles of Respect for Autonomy, Beneficence, and Nonmaleficence

By their very nature, children are in an earlier stage of cognitive development than autonomous adults. It is generally presumed that pediatric patients lack the ability to make autonomous decisions. In contrast, adult patients are presumed to have the ability to make autonomous decisions absent evidence otherwise. As children are not considered fully autonomous, medical decision-making for a child is made by a surrogate, usually a parent. Parents serve as surrogate decision-makers due to a combination of factors: their intimate knowledge of their child’s developing worldview, a presumption that they have a stronger interest in their child’s well-being than the public-at-large, the fact that they play an integral role in the logistics of providing care for a child, and a strong cultural and legal tradition of deference to parental authority.

Parental surrogate decision-making is inherently different than surrogate decision-making for a previously autonomous adult. Surrogate decision-makers for a previously autonomous adult, guided by an advance directive or substituted judgment, are asked to make a decision as the patient would have had they remained

Table 1 The four principles of biomedical ethics [2]

Principle	Brief description
Respect for autonomy	Health practitioners should provide care consistent with the goals and values of the patient receiving care. This should be guided by the decisions of the informed and competent patient whenever possible
Beneficence	Health practitioners should seek to provide care that would be expected to do the most good for their patients
Nonmaleficence	Health practitioners should seek to avoid care that would be expected to harm their patients
Justice	Health practitioners should seek to ensure that only clinically and ethically relevant facts are used as the basis for medical decision-making. Within the broader health care system, the principle of justice suggests that the burdens and benefits of medical care be fairly distributed

autonomous out of respect for the patient's previous autonomy [3]. In contrast, children have never been autonomous; thus, substituted judgment cannot be employed by parents. Instead, parents and healthcare providers are tasked with considering the best interests of the child [4]. Parental authority as surrogate decision-maker is not absolute and may be constrained in some instances where the parental choice would result in harm to the child.

Best Interest Standard

The best interest standard directs decision-makers to focus purely on the child's good, considering the present and future self-regarding interests of the child alone, and make the choice that maximizes benefit (i.e., principle of beneficence) and limits harm (i.e., principle of nonmaleficence) to the child [2–4]. For example, treatment of a relapse with efficacious systemic steroids is likely in the best interest of a child with steroid-sensitive nephrotic syndrome. Treatment with systemic steroids offers the present and future benefits of attaining remission and decreases risk of harms such as peritonitis, thromboembolism, or volume overload. Although systemic steroids are not without potential harm, when balanced against the potential benefit they may provide, it is likely that treatment with systemic steroids is in the child's best interest rather than an alternative choice such as a steroid-sparing agent or no therapy at all. As a result, pediatric nephrologists should guide parents to select systemic steroids, and parents should do so. In other circumstances, systemic steroids may not be in the best interest of the child with nephrotic syndrome. For example, were the child to have more frequent relapses, less or no response to, or greater side effects from systemic steroids, further systemic steroids may not be in a child's best interest as they would not maximize benefit and minimize harms to the child, and a different approach may be warranted.

Although the best interest standard has been long established within medical decision-making and legal contexts, it is subject to certain criticisms. Parents face competing obligations, which may preclude making decisions with a single child in mind [5]. Parents may have other children, a partner, other family members, friends, employers, and others to whom they owe obligations in addition to obligations to their own health and well-being [6]. Furthermore, while the best interest standard is often helpful, it offers limited guidance when the choice of what is "best" is unclear. This may be more common in complex, chronic diseases such as advanced chronic kidney disease where the disease is chronic, incurable, expected morbidity may be significant, costs and other burdens placed on others may be high, and societal resources are limited [7]. Due to these limitations, while the best interest standard works well to guide decision-makers, it does not function well as a threshold for pediatric nephrologists to seek state or institutional power to override a parental decision [8].

The Harm Principle

Parents may place different values on benefits and burdens of treatment options compared to medical professionals. This means well-meaning parents and pediatric nephrologists could arrive at different interpretations of what is “best” for a child. Rather than guiding parents and clinicians toward making the best decision, the harm principle seeks to define what choices should be considered unacceptable for parents to make, thereby justifying seeking institutional or state power to override those choices [9]. Parents are not required to make the very “best” decision for their child in all situations. Instead, they should not be permitted to make decisions (usually a refusal of treatment) in which serious, imminent harm is expected to occur to the child, an alternative could avoid that harm, there are no reasonable alternatives to overriding the parents, and there would be broad social agreement with providing the recommended therapy in this and other similar situations [9–11].

Seeking State Intervention

Wherever possible, clinicians should seek to work with patients and their families even in the setting of disagreement over treatment choice. This can involve repeated meetings, providing additional education, seeking support from a multidisciplinary team, pursuing mediation, and other pursuits. In rare circumstances, these approaches may fail, resulting in an intractable conflict. In cases where nephrologists feel a parent’s choice for their child should not be respected, state intervention should be sought to overrule parental authority and provide consent for treatment on behalf of the child. As a general rule, nephrologists should seek state intervention only as a last resort, as court involvement to order treatment over parental objections represents a serious challenge to parental authority and may permanently alter a family’s future interactions with medical providers.

The exact process of seeking state intervention varies between countries and regions. This is impacted by at least three factors. First, legal precedent justifying state intervention differs between countries. For example, in the United States, state intervention is justified when a parent refusal meets a threshold of harm (exceeding the harm principle) [9, 12]. In contrast, in England state intervention is justified if a judge believes a parent’s decision is not in the child’s best interests [12]. This means in certain circumstances, state intervention may be appropriate in England (choice not in child’s best interest), but not in the United States (choice exceeds harm principle) [12]. Second, the availability of resources varies significantly between countries. For example, in low- and middle-income countries standards justifying state intervention may be based upon the best interest of the child or harm; however, in practice they are not enforced as there may be insufficient resources to pay for efficacious treatment that is in the best interest of the child [13–15]. Third, the harm principle requires public consensus (test of publicity) that overruling parental refusal is justified [9]. This means it is possible state intervention may be justified in one country, but not in another based on variation in public consensus between countries and regions.

Recognizing the complexity of nephrologists seeking state intervention to overrule parental authority, there remain cases in high-income countries where it is clearly indicated. For example, parental refusal of recommended dialysis for a 6-year-old with stage 3 acute kidney injury from diarrhea associated hemolytic uremic syndrome, clear indications for acute dialysis, and a high likelihood of renal recovery, and no known comorbidities may violate the harm principle. Refusal of dialysis treatment places the child at clear risk for severe, imminent, and preventable harm compared to the alternative (dialysis). If a parent refused dialysis in this situation, a pediatric nephrologist could apply the harm principle and explore whether the parent’s choice can be respected or whether their authority should be abrogated and consent for treatment be provided by the state. Similarly, a nephrologist should seek state intervention to overrule parental refusal of all treatment for grade IB acute cellular-mediated rejection of kidney allograft for their child. Refusal of treatment places the child at clear risk for serious, preventable, and imminent harms (e.g., loss of kidney allograft) compared to the alternative (immunosuppressive medications). The risks of immunosuppressive medications do not exceed those of no treatment. There is not a less intrusive alternative to seeking to overrule parental refusal, and such a decision could be generalized to similar cases, and most parents would agree intervention is reasonable. Additional examples that likely exceed the harm principle threshold for seeking state intervention include parental refusal of immunosuppression for a child with active class IV lupus nephritis, refusal of all therapy for a child with idiopathic nephrotic syndrome, and refusal of antibiotics for spontaneous bacterial peritonitis for a child with nephrotic syndrome. In contrast, parent refusal of antihypertensive medication due to a preference to focus on lifestyle modifications for a child with longstanding stage 1 hypertension may not be in the child's best interest but is unlikely to exceed the threshold of the harm principle. Parental refusal may place the child at risk for development of cardiovascular or kidney disease, but the significance and imminence of this risk is less clear. Additionally, the parent's preferred treatment of lifestyle modifications could also decrease those risks, albeit less so than with additional medications. Finally, it is unlikely that state intervention could be generalized to similar situations or that most parents would agree that state intervention was reasonable. In each case, a decision to seek state intervention to overrule parental authority requires due consideration and deliberation, and likely the involvement of a multidisciplinary care team potentially including social work, ethics consultant, and others [16].

Decision-Making in Special Populations in Pediatric Nephrology

Infants

Historically, initial attempts at neonatal and infant dialysis were associated with poor outcomes, mortality, and morbidity. As a result, a consensus emerged within the pediatric nephrology community that there was no moral obligation to provide dialysis treatment to infants if parents did not want to pursue such treatment [17–19]. Due to improvement in knowledge and technology over the last 40 years, outcomes have markedly improved. An analysis of the North American Pediatric Renal Trials and Collaborative Studies cohort (NAPRTCs) between 2000 and 2012 demonstrated a long-term survival rate of 78.6% among newborns for whom dialysis was initiated at less than 1 month of age [20]. Similar findings have been reported across a wide variety of studies [10]. Yet, while international surveys of the medical community suggest that the vast majority of pediatric nephrologists will offer dialysis to at least some infants with end stage kidney disease, the surveys also demonstrate that a majority of nephrologists feel it remains permissible for parents to decline such therapy for infants less than 1 year of age [21–23].

Application of the ethical frameworks discussed earlier in this chapter highlight two aspects of the decision-making process for these infants that require scrutiny. First, should the improvement in the outcomes for infants treated with kidney replacement therapy constrain the range of treatment choices for parents when examined through the lens of either the best interest standard or the harm principle? [10, 19, 23, 24] Second, it is important to recognize that medical professionals are more comfortable withholding life-sustaining treatments in neonates than in older infants and children [25]. An informal survey of members of the European Peritoneal Dialysis Working Group demonstrated this phenomenon, in which 55% of respondents felt that it was acceptable to respect a parent's wish to refuse dialysis for an otherwise normal neonate (<30 days of age), whereas only 26% felt parental refusal was acceptable for an infant 1–12 months of age [23]. These results suggest pediatric nephrologists and other clinicians consider neonates as a distinct group from older infants [25]. Yet, is such a distinction ethically warranted? As discussed above, a central aspect of the principle of justice is that only clinically and ethically relevant aspects of a patient should be considered when determining whether or not to provide a given treatment. It is not clear that this distinction in decision-making between newborns and older infants would qualify. Further details on the ethics of infant dialysis are discussed in ▶ Chap. 77, "Infant Dialysis."

Adolescents

While most children are presumed to lack the capacity to make autonomous decisions, this capability develops throughout childhood. Adolescence marks a period of transition from childhood to adulthood characterized in part by developing the capacity for autonomy. This process varies significantly from patient to patient, likely continues into the patient's 20s, and is further complicated as there is not a singular aspect of cognitive or emotional development that clearly corresponds to "adulthood" or the ability to make "autonomous" decisions [26].

While different countries may recognize adulthood at different ages in various cultural and legal contexts, all pediatric nephrologists face a dilemma of balancing respect for an adolescent's developing autonomy, parental authority, and acting in the adolescent's best interest [27–29]. As patients mature, it is appropriate to seek out and become more deferential to their values and goals, and to incorporate them into shared decision-making. This is supported by two ethically relevant considerations: (1) Respect for autonomy can encompass respect for the developing autonomy of an adolescent. (2) The adolescent's goals and values should help shape assessments of beneficence and nonmaleficence for the patient. This approach is codified in some countries. For example, in England, adolescents aged 16–17 are assumed to have competence to provide consent for medical treatment independent of parental consent [30]. Additionally, certain adolescents under age 16 may provide consent if they are found to demonstrate Gillick competence [30]. This requires demonstrating sufficient maturity and intelligence to understand and appraise the nature and implications of the proposed treatment, including the risks and alternative courses of actions. Importantly, Gillick competence permits an adolescent to provide consent for medical treatment without parental consent but does not permit any adolescent (less than 18 years) to refuse a treatment that is in their best interest [30]. In this way, a "Gillick competent" adolescent could provide consent to receive treatment such as antibiotics for spontaneous bacterial peritonitis but could not refuse it as the treatment is clearly in the adolescent's best interests.

In some circumstances, an adolescent patient may refuse a recommended treatment that, in the eyes of the clinician, is clearly the most beneficial course of action. While a strict ethical analysis of a given circumstance might support treating a pediatric patient over their objections, this is practically challenging, if not impossible, if an adolescent with kidney disease actively dissents [28]. This may be particularly true for treatments that are chronic or those that require some level of patient participation such as dialysis or maintenance of kidney transplant [28]. Forcing an adolescent to receive treatment over their own objection risks obliterating all therapeutic alliances and trust between patient and care team, and the harms of such an approach likely will outweigh the benefits. Instead, pediatric nephrologists should pursue a more comprehensive, multidisciplinary approach. This should include continued engagement with the adolescent and their family to better understand their concerns. It is possible that clinicians' perception and grading of benefit and burden of an intervention may be very different than that of the patient themselves [28]. Additionally, pediatric nephrologists should solicit input from allied professions such as social work, psychology, palliative care, ethics, religious counsel, or others in an effort to reach consensus on the course of action moving forward.

Considerations of Justice

Justice is a complex principle concerned with fair, equitable, and appropriate treatment in light of what is owed to persons [2]. The minimal requirements of justice in western bioethics are reflected in Aristotle's principle of formal justice which holds that equals must be treated equally and unequals must be treated unequally [2]. As applied to children with kidney disease, it means that two children with the same kidney disease should be treated similarly unless there is a morally significant difference between them. Although intuitively appealing, this interpretation of justice is challenged by realities encountered by pediatric nephrologists including expensive treatments, impact on caregivers and families, and systemic injustices among others. These challenges impact individual patient encounters, work with communities and within a healthcare system, and the role of nephrologists as global citizens.

Justice and Resource Constraints

Medical care has a cost which must be paid for it to continue to be available [31–33]. While experienced differently depending on healthcare system and socioeconomic status, nearly all patients and their families must contend with the cost of medical treatments. One area where high costs are experienced is expensive drug treatments. Most treatments for children with kidney disease require the prescription of increasingly expensive medications. Inability to pay for such treatments increases the risk of poor outcomes such as the progression of chronic disease and transplant graft failure [34, 35]. Among the most expensive medications are new biologic agents which offer the possibility to control, but not cure, conditions and require a lifetime of administration [36]. The most expensive of these cost several times the per capita gross domestic product of even the most affluent nations [33, 36]. Even acknowledging the considerable costs of drug development and the role of collective resources such as group health insurance or nationalized health care to spread the costs of a treatment over a population, such a model of expensive treatment is not sustainable on a large scale [35, 36]. The UN Universal Declaration of Human Rights classifies medical care as a basic necessity [37]. If medical care is a basic necessity, excessively high drug prices are unjust because they exploit the vulnerability of the patient and their family [33].

Recommending, requesting, or requiring treatment without a mechanism to pay for it is an additional stress on decision-making and raises ethical concerns of fairness. Disease and the expense of treating it are not distributed equally, nor are children generally viewed as being responsible for their state of health. As a consequence, fairness may dictate societal support to mitigate the expense of medical care for children. Recognition of this tension suggests pediatric nephrologists should be conscious of the expense of treatments and openly discuss this with families, seek to improve access to preventative and cost-efficient treatments, and work with allied professions, patients, and families to advocate and demand for resources for particular patients and more generally for implementation of public health policies to support children with kidney disease and their families [36, 38].

An example of the efficacy of such advocacy was the efforts nephrologists and others to highlight the limited access to dialysis treatments in the United States in the 1960s and 1970s driven by cost. Public outrage at the subsequent rationing of dialysis treatment led to incorporation of the provision of dialysis treatment within the Medicare entitlement in the United States. In effect, end stage kidney disease became the only disease for which there is nationalized healthcare available for American citizens. While a useful example, advocacy alone cannot address the significant expense of treatments for pediatric kidney disease experienced throughout the world, particularly in lower- and middle-income countries where resources to treat disease are more constrained.

Denial of access to medical treatment based on ability to pay raises serious concerns of justice, even if practically they may be unavoidable. One approach advocated in these circumstances is to utilize the principles of procedural justice to develop clear, explicit procedures for considering decision-making for every child [15, 39–41]. Specifically, a fair process requires that four conditions be met: publicity, relevance, revision and appeal, and regulation [41]. When established, procedures for allocating scarce medical goods must be specific to a country or broader region to account for varying levels of available resources and social supports. These procedures should include stakeholders (patients, caregivers, nephrologists, and community leaders) in their development, be informed by relevant evidence and ethical principles, and offer the opportunity for patients and caregivers to appeal a decision.

Examples of such policy are the explicit systems of kidney allograft allocation developed by many individual countries and groups of countries. These systems define the criteria by which kidneys are allocated (waiting time, age, severity of illness, etc.), are publicly available, and strictly allocate kidneys by these criteria. While such frameworks cannot guarantee all children receive a desired treatment, they offer each child and their family a fair and open process for decision-making.

Caregiver Burden

Pediatric chronic kidney disease impacts not only a child, but also their family and community. In a systematic review of studies of caregiver experience in pediatric CKD, Tong and colleagues described the experience as “pervasive and profoundly negative experience” [42] . Going further, Ojo described development of ESKD as “a life-changing tragedy for the child, their family, and their community” [43]. These descriptions reflect the significance and import of the burdens placed on caregivers of children with CKD, particularly mothers. Caregiver burden is best thought of as a multidimensional construct reflecting an individual’s perception of overload during the caregiving process in one or more of four perspectives: physical, psychological, social, and financial [44]. While caregiver burdens may be greatest for younger children and those with more advanced kidney disease, these impacts may be experienced caring for children in all levels of CKD [7, 45–51]. At its most significant, caregivers (particularly mothers) experience extraordinary burdens which, in some circumstances, exceed their available physical, financial, or psychological resources, so they are unable to meet their duties to themselves or to others for whom they are responsible [6, 7, 45, 46]. This experience has been reported by caregivers throughout the world and is influenced by the available social resources within each country [7].

In light of the severity of the burdens experienced by at least some caregivers, it seems cruel and unjust for nephrologists not to discuss expected burdens with parents. This raises challenging ethical questions including how clinicians and parents could or should incorporate considerations of caregiver burden into medical decision-making for a child with chronic kidney disease. More directly, these cases highlight a situation where a choice may be in the best interest of a child, but not in the best interest of other children in the family, the family as a whole, or the community. As described earlier, ethical frameworks of pediatric decision-making focus on the best interests of the child alone when weighing treatment options [52]. Such frameworks seek to highlight the primacy of the needs of the child and to protect the child from exploitation by those more powerful and hold that a child’s basic needs must always be met [4, 5, 9]. While intuitively appealing to pediatricians, such a stance overlooks that parents, siblings, and other caregivers are also moral agents with finite resources and pragmatically that even in the wealthiest countries public resources may be insufficient to meet the needs of children with kidney disease and their families [7]. Yet, it seems unfair for societal failures to support children and families to fall exclusively on the vulnerable child with kidney disease. The ethical tension between the needs of the child, other members of the family, and community in a setting of constrained resources does not offer a ready solution. It highlights a gap in the current ethical framework of pediatric medical decision-making. Recognition of this calls not only for advocacy from the pediatric nephrology community to appeal for greater supports for children and their families, but also for nephrologists to recognize the situation their patient and their patient’s families find themselves.

Systemic Injustice

Increasingly, it has been demonstrated that social determinants play a dramatic role in shaping health outcomes for children with CKD and their families. These include societal problems of structural racism, sexism, ableism, classism, and the biases associated with them which are experienced differently by particular individuals and groups within populations [53–55]. In addition, adverse environmental conditions and differences in access to healthcare experienced by particular groups can influence both the emergence and the progression of kidney disease [53]. This insight supports the need for critical reflection in clinical practice and research. For example, the use of race as a prognostic factor or association with an outcome of interest may overlook the impact of racism rather than race in influencing clinical outcomes [56–58]. The intersection between kidney disease and racial, ethnic, gender, and socioeconomic disparities further highlights how particular groups are disadvantaged [59]. Nephrologists should seek to become aware of the impact of social determinants of health and implicit biases and work to dismantle structural causes of inequity that impact the health and well-being of patients, families, and colleagues [55].

Further, it is incumbent upon nephrologists to recognize how historic injustices including colonialism and racism contribute to the inequities resulting in high rates of acquired kidney disease in children in low- and middle-income countries. This is seen through effects of environmental and poverty-related impacts including high prevalence of bacterial, viral, and parasitic infections, limited access to reproductive care, low birth weight, malnutrition, food insecurity, poor sanitation, unsafe water supply, and pollution all of which contribute to the development of kidney disease [38, 53, 60–62]. These injustices highlight the need for increased and sustained efforts to support children with kidney disease and those caring for them in the developing world as an integral part of pediatric nephrologists' roles as advocates and global citizens. Examples of such efforts include the training of and partnerships with pediatric nephrologists and allied professions practicing in low- and middle-income countries to better meet the needs of these populations [63]. Other examples include collaborative efforts such as the International Society of Nephrology's 0 by 25 initiative and the Saving Young Lives initiative supported by the International Pediatric Nephrology Association, International Society of Nephrology, International Society for Peritoneal Dialysis, and the Sustainable Kidney Care Foundation which focus on reducing child mortality and morbidity from acute kidney injury by establishing and maintaining centers for care of acute kidney injury and efforts to develop and maintain kidney disease registries [35, 38, 64–67].

Health Practitioner Bias

High-quality pediatric nephrology care requires tailoring recommendations to the needs of each individual child with kidney disease and their family. Such an approach may be as simple as prescribing a liquid form of a medication for a patient who cannot swallow pills or as complex as weighing the relative benefits of various therapies to a patient and family who face significant financial, social, and logistical challenges impeding optimal treatment. One challenge of individualized recommendations is that they can be influenced by the clinician's own perception of the challenges that a patient and their family face, and these perceptions can be influenced by practitioner bias. Bias is often characterized as: (1) explicit bias, in which the individual is aware of their feelings and attitudes that can influence their behavior, and (2) implicit bias, in which the individual is unaware of their feelings and attitudes that can influence their behavior. Bias of either type violates the principle of justice by incorporating factors that are not ethically relevant (ethnic background, class status, etc.) into the medical decision-making process.

Unfortunately, implicit bias is endemic in society and has been demonstrated in the medical care of nonmajority populations worldwide [68]. Pediatric providers are not immune from this phenomenon. Studies have demonstrated significant rates of implicit bias directed against both minority adults and children among practitioners [69, 70]. Such bias has been demonstrated to impact treatment recommendations in theoretical scenarios of pediatric care [71]. Although much of the research on implicit bias has been focused on bias against minority populations, implicit bias has the potential to affect the care of other disadvantaged groups.

Example: A Nonadherent Adolescent with Hypertension and Proteinuria[1]

A 16-year-old African American male receives care in an urban American pediatric nephrology clinic for hypertension and proteinuria in the setting of uncontrolled type 2 diabetes and obesity. He is a former preterm infant with low birth weight. Both of his parents have hypertension, diabetes, and obesity. After years of follow-up, the young man has made little progress in improving blood pressure or blood sugar control or losing weight. His physician expresses frustration due to the patient and his family's presumed nonadherence with recommendations and the resulting progression of disease.

While adherence with medical recommendations is critical in a successful therapeutic plan for any child, nonadherence is very common among children with chronic kidney disease, and pediatric nephrologists' perceptions of adherence may also be influenced by implicit biases including those related to race and socioeconomic status [54, 69, 72, 73]. Further, adherence with recommendations may be related to patient and family trust in their physician and more broadly in the medical establishment. In the United States, mistrust of the medical team within certain communities, including African Americans, may be well-founded and based upon historic episodes of exploitation of those communities.

In addition, while concerns for failure to adhere to diet, lifestyle, and medication recommendations may be perceived as a choice by the patient and/or their family and consequently that the adolescent and his family bear all or much of the responsibility for the patient's disease, we would urge nephrologists to recognize many systemic factors that may also be contributing. These factors include the effects of racist policies such as housing segregation that created unsafe neighborhoods and poverty, which can impact the ability to afford medications. The effects of these policies intersect with environmental injustice of lack of access to healthy food, food insecurity, and unsafe neighborhoods which limit access to healthy foods and opportunities for aerobic exercise. Further systemic injustice related to racism has been shown to contribute to preterm birth and low birth weight and lower nephron mass entailing an increased risk of hypertension, proteinuria, and progression of kidney disease.

The purpose of this example is not to deny moral agency to this patient and his family, but to highlight how unfair systemic forces outside the patient's control may also influence situations our patients find themselves in. This reinforces the importance of nephrologists maintaining an open mind, seeking to build supportive relationships, and working to advocate both for our individual patients and to address and dismantle the systemic forces that negatively influence our patient's health.

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[1] The authors practice in the United States, and therefore, the example used reflects practice within that country. Although it is our firm belief that similar issues could be illustrated through other examples set in other countries, we lack the requisite cultural knowledge to do so accurately.

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Drug Dosing in Abnormal Kidney Function in Children

Mara Medeiros and Guido Filler

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Abstract

As more than 60% of drugs are cleared by the kidneys, knowledge of drug handling and drug dosing in children with abnormal kidney function is essential. However, predicting drug clearance of drugs by the kidneys is not so straightforward. Very few drugs are actually cleared by glomerular filtration as this process requires that the drug to be unbound to plasma protein and an approprite size. Most drugs are actively transported into the lumen of the tubule and functionally, intact nephron endowment would be required for accurate drug clearance. Unfortunately, ways to assess nephron endowment are not clinically practical and therefore

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glomerular filtration rate (GFR) remains the best surrogate marker of nephron endowment.

In this chapter we will discuss developmental changes of drug transport, how children handle drugs with normal and impaired kidney function, and general principles of drug dosing in children with chronic kidney disease (CKD). We will also cover drug dosing during renal replacement therapy.

Keywords

Pharmacokinetics · Pharmacodynamics · Drug transporters · Ontogeny · Filtration fraction · Nephron endowment · Creatinine · Cystatin C · Acute kidney injury · Drug toxicity · Antibiotics · AKI · Dialysis · Kidney replacement therapy

Abbreviations

AAG	Acid glycoprotein
AKI	Acute kidney injury
APD	Automated peritoneal dialysis
CAPD	Continuous ambulatory peritoneal dialysis
CAVHF	Continuous arterio-venous hemofiltration
CKD	Chronic kidney disease
CRRT	Continuous renal replacement therapies
CVVH	Continuous veno-venous hemofiltration
CVVHD	Continuous veno-venous hemodialysis
CVVHDF	Continuous veno-venous hemofiltration
CysC	Cystatin C
ERPF	Effective renal plasma flow
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
HD	Hemodialysis
IHD	Intermittent hemodialysis
KDIGO	Kidney Disease: Improving Global Outcomes
PD	Peritoneal dialysis
RRF	Residual renal function
SCUF	Slow continuous ultrafiltration
SLED	Sustained low-efficiency dialysis
V_d	Volume of distribution

Background

The pattern of drugs used in children is different from adult populations because diseases and pathogen susceptibility undergo change with age. There are also substantial developmental changes of drug disposition [1]. There is a perception that drugs are rarely used in children. However, based on 1999–2014 National Health and Examination Survey, 21.9% of children and adolescents used any prescription drug in the past 30 days, while 8.5% used more than two drugs during the study period from 2011–2014. While the use of antibiotics and antihistamines decreased compared to the period 1999–2002, antihypertensives, asthma medications, attention-deficit hyperactivity disorder medications, proton pump inhibitors, antipsychotic, and contraceptive medications increased [2].

Off-label drug use is more prevalent in the pediatric population, referring to the use of pharmaceutical drugs for a different application, age group, dosage, or route of administration than their specific approval. This poses a problem as the ontogeny (developmental changes) of drug disposition has not been studied. For some specialized agents like antihypertensives, there are few pediatric formulations and for others, there are no pediatric studies to recommend a dose, therefore the adult dosage is extrapolated to children [3].

When using drugs in children it is important to consider all the factors involved in the drug-response relationship, including those related to pharmacokinetics and pharmacodynamics as well as prescription, adherence, and formulation details. There are important changes in drug bioavailability related to age, due to different pharmacokinetics (the absorption, distribution, metabolism, and elimination of drugs) and pharmacodynamics (the effects on the body), as well as developmental changes in body composition, distribution sites, gastrointestinal function, metabolic capacity, renal capacity, drug bioavailability, and metabolism. In general, the therapeutic index narrows in children [1, 4].

Drug metabolism can be performed by phase 1 or phase 2 reactions [5]. Phase 1 or nonsynthetic reactions comprehend oxidations, reductions, and hydrolysis and are mediated by enzymes mainly from p450 cytochromes, alcohol dehydrogenases, and aldehyde oxidases. Phase 1 reactions are involved in transforming drugs to more polar compounds for easy elimination through bile or urine, and inactivation or production of active or toxic metabolites. The expression of the different isoforms of cytochromes changes with age. Phase 2 reactions are also called synthetic and comprehend the conjugation with glucuronide (uridine-5-diphospho-glucuronosyltranspherase, UGT), sulphate (sulphate transpherase, SULT), glutathione (glutathione-S-transpherase), methylation (methyltranspherases), and acetic acid (acetyltranspherase). All of these compounds make more hydrophilic substances to facilitate clearance. Transporters are proteins located in different tissues of the apical and basolateral membranes, to facilitate the movement of compounds within the cell and from one compartment to another. They are crucial in pharmacokinetics because of their role in absorption and distribution of drugs, mainly against concentration gradient. Variations in the activity of transporters are extremely important for drug safety as they change with age, genetic polymorphisms, disease conditions, environmental factors, and drug interactions to modify drug clearance and toxicity [6, 7]. The kidneys express many transporters [8]. There is scarce information about the ontogenetic changes in the kidneys, but some models have been developed to predict toxicity of antibiotics in neonates, in whom the toxicity can vary depending upon postnatal age, gestational age, weight, and comorbidities [9].

Renal Clearance

The kidneys receive 25% of cardiac output beyond 18 months of life and are very active in biotransformation and clearance of drugs. There are substantial developmental changes in kidney function during the first 18 months of life [10]. Renal excretion of drugs relay upon filtration, active secretion/reabsorption, and passive diffusion. Free drug (not bounded to protein) is filtered by the glomeruli and undergoes reabsorption and secretion depending upon the drug transporters present.

Clearance is summarized as follows:

(Unbound drug × glomerular filtration rate (GFR)) + secretion-reabsorption [11].

However, most drugs have plasma protein binding, and their secretion depends on the number of functioning nephrons. Reabsorption of the drug along the proximal and distal tubules and the collecting ducts mitigate the drug's elimination from the body, both by filtration and by active secretion. Although reabsorption is primarily a passive process, it can have a profound impact.

Even though nephron endowment may be more important for drug clearance, we are not able to measure this directly, so we use GFR as a surrogate. Although inulin clearance is the gold standard, its use is challenging, especially in an acute setting; therefore, renal function is typically measured using endogenous markers [12]. Serum creatinine is the most commonly used endogenous marker but its use may be problematic in newborns [13].

Cystatin C [14], a different endogenous marker, may be more accurate for estimating renal function in children. Formulae that incorporate both cystatin C and serum creatinine are more robust in this age group [15]. Beta-trace protein, yet another marker, is believed to be a suitable alternative for newborns [16]. Nevertheless, these markers assess GFR rather than the effective renal plasma flow (ERPF), which would likely provide a better estimate of the kidneys' ability to excrete a drug [17]. For instance, hyperfiltration and changes in ERPF and filtration fraction all affect serum creatinine [18]. We recommend using the latest Schwartz formula, utilizing both cystatin C and creatinine for estimation of GFR [19].

Drug Properties and Dosing in Abnormal Kidney Function in Children

The most evident consequence of CKD is a decrease in the renal excretion of drugs. GFR-based adjustments are required if drugs are excreted through renal pathways per guidelines [20–23]. A less apparent effect of CKD on drug disposition is altered non-renal clearance

[24–27]. This can primarily be attributed to decreases in both hepatic drug metabolism and transporter-mediated biliary drug clearance [28]. Clinicians should avoid assuming that non-renally excreted drugs do not require a dose adjustment. Based on renal function level, Table 1 describes the drug dosing of commonly used antibiotics; Table 2 focuses on other antiorganisms; Table 3 on anticonvulsants; and Table 4 on immunosuppressants, diuretics, and other drugs. These tables are updates on the previous version of this book. Analgesics, antipyretics, and anti-inflammatory drugs can be used with caution, considering the potential nephrotoxicity of some drugs. Paracetamol and dipyrone are the most used analgesics in many countries since the renal toxicity is rare at therapeutic doses.

Drug Clearance in Dialysis

The physicochemical properties of a drug such as molecular size, protein binding, and volume of distribution largely determine its dialyzability. Additional factors specific to the dialysis prescription such as the type of dialyzer and blood/dialysate flow rates can also impact drug removal.

High-flux dialyzers can remove drugs with molecular weights of up to 40,000 Da. In practice, the pediatric nephrologist must ensure that references pertaining to dialytic drug clearance are current. For example, vancomycin (~1,450 Da) is minimally cleared with conventional dialysis but is extensively cleared with high-flux dialyzers (dialytic clearance between 45 and 131 ml/min with high-flux polysulfone dialysis membranes) [29, 30]. Other drugs that exhibit the same characteristic include carbamazepine, cisplatin, daptomycin, fluorouracil, ranitidine, and valproic acid [31].

The degree of plasma protein binding plays a large part in determining the degree to which a drug is removed by dialysis. Those with minimal protein binding are more likely to be removed by dialysis. Further complicating the impact of protein binding, kidney disease itself has been shown to affect the two major plasma proteins, albumin and alpha 1 acid glycoprotein (AAG). While dialysis patients often exhibit hypoalbuminemia, the inflammatory nature of CKD is thought to result in an increased concentration of AAG [32, 33]. Furthermore, uremic solutes that accumulate in CKD are known to increase the free fraction of some drugs, making them more easily dialyzable [34–38]. For example, uremic toxins are known to displace phenytoin from its binding sites on albumin, resulting in a greater free fraction of phenytoin available for removal by dialysis.

The volume of distribution of a drug also has a profound impact on its dialyzability. Drugs that have a small V_d (e.g., <0.7 L/kg) are generally restricted to the blood compartment and therefore more accessible for removal during dialysis. In contrast, drugs with a large V_d are distributed into the tissues and are generally minimally impacted by dialysis. Drugs with a large V_d are also more susceptible to the rebound effect following dialysis. Examples of drugs with a substantial rebound effect include gentamicin and vancomycin. Gentamicin concentrations rebound by up to 25% several hours following the completion of a dialysis session [39]. Vancomycin exhibits a similar rebound as plasma concentrations decrease by 38% immediately following dialysis but are only 16% lower than the predialysis concentration 5 h post-dialysis [40].

An excellent resource for drug dialyzability information is an annual publication by the Renal Pharmacy Consultants titled *Dialysis of Drugs* [31]. Despite the utility of this resource, many drugs lack information on this topic. Using *Dialysis of Drugs*, the authors determined that of all drugs surveyed for modern high-flux dialysis, only approximately 10% had clear evidence demonstrating whether they were dialyzable. An additional 40% were determined to be “likely” or “unlikely” to be dialyzed based on their physicochemical properties, with the remaining 50% of drugs having no information available [27]. Given the large proportion of drugs without data on drug dialyzability [27], it is important for the pediatric nephrologist to recognize the importance of the main factors that determine this characteristic such as size, protein binding, and volume of distribution, in order to rationally dose drugs in pediatric patients on hemodialysis.

Table 1 Drug dosing of commonly used pediatric antibiotics. MW=Molecular weight, h=hour

Drugs	Normal dose/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR < 10	MW	Plasma protein binding	%eliminated by kidney	Elimination t ½ with normal GFR
Aminoglycosides								
Amikacin	15–22.5 mg/kg div Q8h	Q12–18h	Q18–24h	Q48–72h	5.856	<11%	>95	2–3 h
Gentamicin	6–7.5 mg/kg div Q8h	Q12–18h	Q18–24h	Q48–72h	477.6	<30%	>95	5–>100 h
Tobramycin	6–7.5 mg/kg div Q8h	Q12–18h	Q18–24h	Q48–72h	467.5	<30%	>95	2–3 h
Carbapenems								
Imipenem + cilastin	60–100 mg/kg div Q6h	7–13 mg/kg /dose Q8h	7.5–12.5 mg/kg/dose Q12h	7.5–12.5 mg/kg/dose Q24h	Imipenem: 299.3	Imipenem 13–21% cilastin 40%	70	1 h
Meropenem	30–100 mg/kg div Q8h	20–40 mg/kg/dose Q12h	10–20 mg/kg/dose Q12h	10–20 mg/kg/dose Q24h	383.5	2%	70	1 h
Cephalosporins								
Cefaclor	20–40 mg/kg div Q8–12h	Normal	Normal	50% dose	367.8	25%	80	40 min
Cephalexin	25–100 mg/kg div Q6–8h	Normal	Q8–12H	Q12–24H	347.4	10.60%	~100	1–1.5 h
Cefazolin	50-150 mg/kg div Q8h	60%, Q12h	25%, Q12h	10%, Q24h	454.5	74–86%	80–100	2 h
Cefixime	8 mg/kg div Q12–24h	75%	75%	50%	453.4	76–91%	20–35	3–4 h
Cefotaxime	100–200 mg/kg div Q6–8h	35–70 mg/kg, Q8-12h	35–70 mg/kg, Q12h	35–70 mg/kg, Q24h	619.6	31–50%	80	1.4–1.9 h
Cefotiam	50–100 mg/kg				525.6	76–91%	80	0.9–1.2h
Ceftazidime	100–150 mg/kg div Q8h	50 mg/kg q12h	50 mg/kg q24h	50 mg/kg q48h	546.6	17%	80–90	1.8–2.2 h
Ceftriaxone	50–100 mg/kg Q24h	Normal	Normal	Normal	554.6	85–95%	67	6–9 h
Cefuroxime	75–150 mg/kg div Q8h	Normal	Normal dose Q8–12h	Normal, Q24h	424.4	33–50%	95	1–1.5 h
Cefuroxime axetil	20–30 mg/kg div Q12h	Normal	Normal dose Q24h	Normal Q48h	510.5	33–50%	95	1–1.5 h

(continued)

Table 1 (continued)

Drugs	Normal dose/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR < 10	MW	Plasma protein binding	%eliminated by kidney	Elimination t ½ with normal GFR
Glycopeptides								
Teicoplanin	10 mg/kg/dose Q12h × 3 then 3–10 mg/kg Q24h	50% ?	Avoid ?	Avoid ?	1893.7	71.9–80.5%, higher in older children	42–58	21–58 h
Glycopeptides								
Vancomycin	40–60 mg/kg div Q6h	10 mg/kg Q12H	Q18–24H	Single dose of 10 mg/kg, then follow levels	1449.3	55%	80–90	4–11 h
Ciprofloxacin	20–30 mg/kg div Q12h	Normal	Q18H	q24h	331.3	16–43%	30–50	3–5 h
Macrolides								
Azithromycin	10 mg/kg X1 day, then 5 mg/kg Q24h × 4 days	Normal	Normal	Normal	749	7–51%	6	54.5 h
Clarithromycin	15 mg/kg div Q12h	Normal	8 mg/kg Q12H	4 mg/kg Q24H	748	42–70%	20–40	3–7 h
Erythromycin	30–50 mg/kg PO div Q6–8H	Normal	Normal	50–75% Q6–8H	734	73–81%	2–5	1.5–2 h
Nitromidazoles								
Metronidazole	30 mg/kg div Q6–8h	Normal	Normal	4 mg/kg/dose Q8H	171.2	<20%	60–80	6–14

Penicillins								
Amoxicillin	20–50 mg/kg div Q8H	Normal	8–20 mg/kg/dose Q12H	8–20 mg/kg/dose Q24H	365.4	17–20%	60	1–2 h
Amoxicillin + clavulanic acid (dosed by amoxicillin component)	Variable, dependent on formulation 25–50 mg/kg div Q8h	Normal	8–20 mg/kg/dose Q12H	8–20 mg/kg/dose Q24H	602.7	Amoxicillin 17–20% Clavulanate 25%	Amoxicillin 50–70 Clavulanate 25–40	1–1.5 h
Ampicillin	50–400 mg/kg div Q6h	Normal	Q8–12H	Q12–24H	349.4	10–18%	90	1–1.8 h
Other antibiotics								
Clindamycin	PO 20–40 mg/kg div Q6–8h	Normal	Normal	Normal	425	60–95%	10	2–3 h
Doxycycline	2–4 mg/kg div Q12–24 h	Normal	Normal	Normal	444.4	80–85%	23	12–15 h
Trimethoprim/Sulfamethoxazole TMP/SMZ Dosed by TMP component	Variable PO 6–12 mg/kg div Q12h	Normal	GFR15-30 50% dose	GFR<15 Avoid	543.6	TMP 44% SMX 70%	TMP IV 17–42.4 TMP PO 66.8 SMX IV 7–12.7 SMX PO 30	TMP 4–8 h/ SMX 9–12 h
Nitrofurantoin	5–7 mg/kg div Q6h	Avoid	Contraindicated	Contraindicated	238.2	~40–60%	40	0.3–1 h

Table 2 Antifungal, antituberculosis, and antiviral agents

Drugs	Normal dose/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR < 10	MW	Plasma protein binding	%eliminated by kidney	Elimination t 1/2 with normal GFR
Antifungal agents								
Amphotericin B	0.25–1.5 mg/kg Q24h	Normal	Normal	Q24–36H	924.1	90%	2–5	12–40 h
Fluconazole	3–12 mg/kg Q24h	50%	50%	50%	306.3	11–12%	80	15–25 h
Itraconazole	5–10 mg/kg div Q12–24 h	Normal	Normal	50%	705.6	99%	0	Parent: 36/Metabolite: 18
Antituberculosis agents								
Ethambutol	PO 15–25 mg/kg Q24h	Normal	Q24-36 h	Q48h	204.3	20–30%	50	2.5–3.6 h
Isoniazid	10–15 mg/kg Q24h	Normal	Normal	Normal	137.1	10–15%	75–95	2.3–4.9 h
Pyrazinamide	15–30 mg/kg Q24h	Normal	Normal	50–100%	123.1	50%	4	6.7 h
Rifampin	10–20 mg/kg div Q12–24 h	Normal	Normal	Normal	822.9	80%	Up to 30%	3–5 h
Antivirals								
Acyclovir	30–60 mg/kg div Q8h	Normal dose q12h	Normal dose q24h	50% dose q 24 h	225.2	9–33	60–90	2–3 h
Ganciclovir	IV 5–10 mg/kg div Q12–24 h	50% dose q24h	25% dose q24h	25% dose 3 times week post-hemodialysis	255.2	1–2%	80–99	2.5–6.5 h
Indinavir	1050–1500 mg/m^2 div Q8h	Not studied	Not studied	Not studied	613.8	60%	<20%	1.4–2.2 h
Lamivudine	8 mg/kg div BID	Normal Q24h	50% dose Q24H	25% dose Q24H	229.3	<36%	70	0.5–4 h
Valacyclovir	60 mg/kg div Q8h	Normal Q12h	Normal q24h	50% dose q 24 h	324.3	13.5–17.9%	88%	1.3–2.5
Valganciclovir	Once daily using formula	Formula	Formula	Formula	354.4	1–2%	80–90%	2–7 h
Zidovudine	Varied	Normal	Normal	50% dose Q8H	267.2	25–38%	63–95	1–2 h
Oseltamivir	Varied	Normal	50%	25% dose q24h	312.14	3%	63–66%	1.8 h

Table 3 Anticonvulsants and antihypertensive drugs

Drugs	Normal dose/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR <10	MW	Plasma protein binding	%eliminated by kidney	Elimination t 1/2 with normal GFR
Anticonvulsants								
Carbamazepine	10–30 mg/kg	Normal	Normal	75%	236.3	75–90%	1–3%	Single dose 40 h/ maintenance 36 h
Clonazepam	0.05–0.5 mg/kg	Normal	Normal	Normal	315.7	85%	Majority	23–36 h
Ethosuximide	15–40 mg/kg div Q12h	50–75%	50–75%	No data	141.2	<10%	10–20	30 h
Phenobarbital	4–8 mg/kg div Q12–24 h	Normal	Normal	No data	232.2	35–50%	≤ 75	
Phenytoin	5 mg/kg	Normal	66%	50%	252.3	90–95%		
Sodium valproate	10–100 mg/kg (plasma levels 5–100 mg/l)	Normal	Reduce or Avoid	Avoid	166.2	80–90%	~100	13–17
Antihypertensive drugs								
ACE inhibitors								
Captopril	0.3–3.15 mg/kg	50% - Normal	25–50%	25–50%	217.3	25–30%	~65	4–5 h
Enalapril	0.1–0.3 mg/kg	25–50%	12.5–25%	6.25–12.5%	376.4	50–60%	61	35 h
Ramipril	0.1–0.2 mg/kg Q24H	50–100%	25–50%	12.5–25%	416.5	73%	60	>50 h
ARB inhibitors								
Irbesartan	75–300 mg	Normal	Normal	Normal	428.5	90%	~20	11–15 h
Losartan	0.7–1.4 mg/kg	Normal	Normal	Normal	422.9	>98%	~35	1.5–2.5 h
B-blockers								
Atenolol	0.5–2 mg/kg	Normal	50%	25%	266.3	6–16%	100	6–10 h
Bisoprolol	0.2 mg/kg	Normal	50%	Normal	325.4	30%	50	10–12 h
Carvedilol	Low-dose: 0.2–12.5 mg/kg High-dose: 0.4–25 mg/kg	Normal	Normal	Normal	406.5	>98%	~35	7 h
Metoprolol	1–6 mg/kg	No data	No data	No data	267.4	12%	~95	3–4 h (though 2–9.5 reported)
Propanolol	1–4 mg/kg div Q8–12H	Normal	Normal	Normal	259.3	93%	<1	4–6 h
Ca-antagonists								
Amlodipine	0.1–0.5 mg/kg	Normal	Avoid	Avoid	408.9	93%	~95	10–36 (with repetitive dosing, 45)
Nifedipine	0.5–2 mg/kg	Normal	Normal	Normal	346.3	92–98%	70–80	4 h

Table 4 Immunosuppressive drugs, diuretics, gastrointestinal, and other drugs

Drugs	Normal dose/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR < 10	MW	Plasma protein binding	% eliminated by kidney	Elimination t 1/2 with normal GFR
Immuno suppressive drugs								
Azathioprine	1–3 mg/kg	Normal	75%, and increase to q36h dosing	50% and increaes to q48-72 h dosing	277.3	30%	~60%	Parent: 12 min/6-MP: 0.7–3 h
Cyclophosphamide	1–2 mg/kg	Normal	75%	50%	261.1	Parent: 20%/ Metabolite: 60%	~60%	6–9 h
Cyclosporine	3–10 mg/kg Div Q12H	Monitor levels	Monitor levels	Monitor levesl	1202.6	90–98% to lipoproteins	6%	5–18 h
Mycophenolate mofetil	1200 kg/m^2	Normal	Avoid doses >1 g BID	Avoid doses >1 g BID	433.5	97%	>90%	Salt: 12 h/mofetil: 16–18 h
Sirolimus	Loading: 3–6 mg/m^2 Maint: 1–2 mg/m^2	Normal	Normal	Normal	914.2	92%	2.20%	7–20 h
Tacrolimus	0.15 mg/kg	Normal	Normal	Normal	804	99%	1–3%	40–50 h
Diuretics								
Acetazolamide	5 mg/kg/dose	Contraindicated	Contraindicated	Contraindicated	222.2	95%	~100%	2.4–5.8 h
Eplenerone					414.5		~67%	
Spironolactone	1–5 mg/kg	Dose q24h	Dose q24h	Avoid	416.6	91–98%	Majority	17–20 h
Gastrointestinal drugs								
Omeprazole	0.5–1 mg/kg	Normal	Avoid	Avoid	345.4	95%	77%	0.5–1 h
Ranitidine	PO 4 mg/kg	Normal	50%	50%	314.4	15%	50–100%	2–3 h
Spasmolytics								
Oxybutynin	<5 year:0.2 mg/kg/dose B-TID >5 year:5 mg B-TID	Avoid ?	Avoid ?	Avoid ?	357.5	>99% alpha-1 acid glycoproteins	<0.1%	7–18 h

For the generation of the tables, we used the following references:

- Lexi Comp Pediatric and Neonatal Dosage Handbook 20th ed. copyright 2013
- on-line Lexi Comp accessed November 2020
- eCPS accessed Jul 2014

Drug Removal During Intermittent Hemodialysis

Hemodynamically stable patients with poor renal function may be suitable candidates for intermittent hemodialysis (IHD). The excretion of renally cleared drugs is related to the residual renal function (RRF) of the patient. As such, only the dialyzability of a drug is considered when estimating dose adjustments for the IHD population. The RRF of the patient determines whether dose reduction or supplement dosing is required [41]. Since there is a lack of pediatric data, current recommendations on drug dosing in IHD patients are based largely on studies conducted in adults. Drug levels post-IHD may also guide treatment.

In 2011, the Kidney Disease Improving Global Outcomes (KDIGO) group held a conference to investigate the impact of renal replacement therapies on drug disposition. The following recommendations for drug dosing in IHD patients were developed: [20]

1. The drug dose should be given after the hemodialysis session (D_{HD}) to maintain active levels prior to subsequent dosing.
2. A supplemental dose should be given (D_{SUP}) in addition to the dose adjusted to renal failure (D_{FAIL}) to compensate for the fraction cleared through dialysis (Fr).

$$D_{\mathrm{HD}} = D_{\mathrm{FAIL}} + D_{\mathrm{SUP}}, \quad \text{where}$$

$$D_{\mathrm{SUP}} = \mathrm{Fr}(D_{\mathrm{START}} - D_{\mathrm{FAIL}})$$

One noteworthy limitation to the KDIGO primary recommendations is their derivation from low-flux nonsynthetic dialysis systems. Accordingly, they advise that the supplemental dose (DSUP) be increased for high-flux synthetic and/or high diffusive membranes.

Another important consideration in the IHD population is that the RRF, while presumed to be near zero, is not necessarily negligible. As a result, the predicted drug clearance in such patients may be underestimated [41]. For medications with a narrow therapeutic index, such as the aminoglycosides or vancomycin, therapeutic drug monitoring is indicated [20, 41]. When monitoring the plasma levels of such drugs, the following formula may be used to calculate the supplemental dose to be given, if requiered:

$$\text{Supplemental Dose} = (C_{\mathrm{DESIRABLE}} - C_{\mathrm{OBTAINED}}) \times V_d,$$

where $C_{\mathrm{DESIRABLE}}$ is the desired plasma concentration of the drug, C_{OBTAINED} is the concentration measured, and V_d is the volume of distribution.

Drug Removal and Dosing During Continuous Renal Replacement Therapies

Continuous renal replacement therapies (CRRT), where the fluids and solutes are continuously removed, are preferred for patients with acute kidney injury (AKI) or sepsis, who are unstable and in critical care units [42]. There are various techniques and strategies, including:

- Continuous arterio-venous hemofiltration
- Continuous veno-venous hemofiltration (CVVHF) [mostly convective clearance]
- Continuous arterio-venous hemodialysis, continuous veno-venous hemodialysis (CVVHD) [mostly diffusive clearance]
- Continuous arterio-venous hemodiafiltration (CAVHDF)
- Continuous veno-venous hemodiafiltration [mix of diffusive and convective clearance]
- Slow continuous ultrafiltration (SCUF) [mostly convective clearance]
- Sustained low-efficiency dialysis (SLED), sustained hemodiafiltration (S-HDF),
  and extended daily dialysis (EDD)

There have been a few studies examining the kinetics of various antibiotics used pediatric critical care units using systems rarely used in children [43–45]. The modality itself, the ultrafiltrate, the dialysate flow rates, the filter, and the patient's RRF may each have a significant impact on a drugs' pharmacokinetics [46].

Physicians often equate the drug clearance of all of the above-mentioned CRRT modalities at low rates of dialysate/ultrafiltrate production (<1 L/h). However, as the rate of ultrafiltrate/dialysate flow increases the difference between them, it becomes more apparent that dialytic-based mechanisms have the lowest solute clearance for a given flow rate.

Drug Clearance of CRRT Modalities : CVVHF > CVVHDF > CVVHD

The removal of drugs with CRRT is understudied, largely due to the vast number of drugs, the various modalities of CRRT, and the relatively small number of pediatric patients per CRRT modality. In general, a drug with a large V_d will be minimally impacted because of the low proportion of the total drug in the blood compartment. Total body water is 65–80% of the body weight in a child. Thus, a drug distributed well to all body compartments, would have a V_d of 0.7–0.8 L/kg. A drug with a V_d >0.8 L/kg would likely have additional tissue binding and CRRT would most likely remove little of the drug. Only the unbound fraction of a drug present in plasma water can be removed by CRRT.

Drugs with a high plasma protein binding (over 80%) including many antibiotics are poorly removed by CAVHD and CVVHD (mostly diffusive clearance), and only moderately cleared by CAVHDF and CVVHDF, as these have equal convective and diffusive clearance mechanisms. Many factors may affect unbound drug concentrations in critically ill children, including blood pH, heparin therapy, hypo-albuminemia, and the presence of fatty acids [47]. The reported plasma protein binding in healthy volunteers may substantially differ in critical care patients [48]. In summary, data on the dosage adjustment of drugs and their dialysability are limited because of a lack of clinical studies [48]. Existing guidelines mainly address antibiotics [48, 49]. In patients with AKI treated with CRRT, drug underdosing may lead to inadequate levels (especially for antibiotics), while overdosing may result in avoidable toxicities, which are often misdiagnosed as adverse drug reactions [47].

Drug Removal by Peritoneal Dialysis

Peritoneal dialysis (PD) is the preferred renal replacement therapy in pediatrics, particularly in small children. Using this technique, fluid and solute exchange occurs across the peritoneal membrane. The procedure may be performed throughout the day over the course of several hours, relying on gravity to force drainage (continuous ambulatory peritoneal dialysis [CAPD]) or through an external device that automatically cycles while the patient is asleep (automated peritoneal dialysis [APD]) [50]. While PD shares many of the principles of hemodialysis listed above, there are several notable drawbacks. The most clinically important difference is that drug clearance is less efficient through PD than through HD. A drug that is dosed appropriately for HD patients may be supratherapeutic for the PD population [51]. With the clearance capacity of PD in mind, the KDIGO group suggested that drug dosing in PD patients can be adapted from the dosing recommendations for patients with eGFR < 15 ml/min [20]. In effect, most PD patients may not require supplemental drug doses compared to other forms of renal replacement therapy.

One caveat, however, is that dosing may change between PD prescription modalities. Very few centers perform tidal dialysis and little data on drug clearance are known. CAPD provides a longer period of consistent drug removal compared to APD, while the latter provides a higher flow rate over a shorter time frame. Consequently, the drug-dosing regimen may differ depending on the PD modality. This necessity for changing drug dosing has been established for numerous drugs including vancomycin [52], cefazolin, and tobramycin [53]. Much like when estimating drug clearance in hemodialysis patients, a clinician must remain aware of the individual’s RRF, because it can result in subtherapeutic dosing.

Another drawback of PD is peritonitis. In 2012, a consensus guideline released by Warady et al. suggested that the intraperitoneal (IP) route be used for antibiotic treatment administered for bacterial peritonitis [54]. Many of the above-mentioned factors concerning drug dialyzability also apply when dosing drugs via IP administration. While there is a paucity of data in children with regard to drug clearance following IP administration, ongoing research is attempting to reconcile pediatric and adult data.

Nephrotoxicity

The kidneys are able to concentrate and eliminate endogenous and exogenous toxic compounds. In some drugs, renal toxicity can be predicted with models that evaluate in vitro necrosis and apoptosis. There is no consensus definition of drug nephrotoxicity, making it difficult to diagnose, report, and compare outcomes. In nephrotoxicity, all nephron segments can be damaged, and there are acute and chronic phenotypes. The definition of acute kidney injury considers serum creatinine and urinary volume, however, sometimes the tubular damage is more important and serum creatinine alone does not allow case detection. Metha and coworkers proposed a phenotype standardization for drug-induced kidney toxicity, which can be presented in one of four phenotypes: acute kidney injury, glomerular disorder, tubular disorder, or nephrolithiasis/crystalluria [55]. A single drug can cause more than one damage phenotype and both, neonates and hospitalized patients are more susceptible to drug-induced renal toxicity.

Conclusions

More than 60% of drugs are cleared by the kidneys and while most are actively transported into the lumen of the tubules, very few drugs are cleared by glomerular filtration due to molecular size and no plasma protein binding. Accurate drug clearance requires intact nephron endowment and accurate renal function measurement.

During acute kidney injury, CKD or ESRD, drug dosing is a dynamic process that is dictated by drug levels, renal replacement therapy, or residual renal function. Drug dosing in patients with renal replacement therapy is determined by the treatment modality. Clinicians should be aware that CKD can affect the metabolism of non-renally excreted drugs.

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Part XII

Addendum

Normal Reference Values

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F. Cano, M. Gajardo, Q. Shen, Edward Nehus, and B. Dixon

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Biochemistry*

	Age range	Male	Female
Alanine aminotransferase (ALT, U/L) [1]	0 to <1 year	5–33	5–33
	1 to <13 years	9–25	9–25
	13 to <19 years	9–24	8–22
Alkaline phosphatase (ALP, U/L) [1]	0 to <15 days	90–273	90–273
	15 days to <1 year	134–518	134–518
	1 to <10 years	156–369	156–369
	10 to <13 years	141–460	141–460
	13 to <15 years	127–517	62–280
	15 to <17 years	89–365	54–128
	17 to <19 years	59–164	48–95
Aspartate aminotransferase (AST, U/L) [1]	0 to <15 days	32–162	32–162
	15 days to <1 year	20–67	20–67
	1 to <7 years	21–44	21–44
	7 to <19 years	14–35	13–26
Beta-2-microglobulin (B2M, mg/L) [1]	0 to <3 months	1.91–4.70	1.89–5.81
	3 months to <2 years	1.31–4.54	1.31–4.54
	2 to <19 years	1.19–2.25	1.19–2.25
Creatinine kinase (U/L) [2]	0–90 days	29–303	43–474
	3–12 months	28–162	27–242
	13–24 months	31–152	25–177
	2–10 years	31–152	25–177
	11–14 years	31–152	31–172
	15–18 years	34–147	28–142
Cystine (leukocyte cystine content) [3]	All ages	<0.2 nmol 1/2 cystine/ mg protein	<0.2 nmol 1/2 cystine/ mg protein
Cystine [3]: In patients with nephropathic cystinosis, the values exceed 2.0 nmol of half-cystine per milligram of protein. Cystine content is expressed in units of half-cystine because initial methods of quantification involved a reduction of cystine followed by an assay for cysteine			
FGF-23 (C-terminal FGF23 concentration, RU/ml) [4]	≤1 year	43–324	43–324
	2 years	39–235	39–235
	3 years	36–192	36–192
	4 years	34–163	34–163
	5 years	33–145	33–145
	6 years	33–135	33–135
	7 years	34–129	34–129
	8 years	35–126	35–126
	9 years	36–126	36–126
	10 years	37–128	37–128
	11 years	37–132	37–132

(continued)

	Age range	Male	Female
	12 years	37–136	37–136
	13 years	36–139	36–139
	14 years	35–140	35–140
	15 years	34–136	34–136
	16 years	32–127	32–127
	17 years	29–114	29–114
	18 years	26–100	26–100
	≥19 years	22–85	22–85
FGF-23 [5]: Median range in healthy children decreases with age from 105 RU/ml (≤1 year) to 50 RU/ml (≥19 years). In children with CKD and a median age of 11.7 years, FGF23 levels increased with higher CKD stage:Stage 2 (GFR ≥ 60 ml/min/1.73 m^2), median FGF23 (IQR) was 93 [73, 140]Stage 3a (GFR = 45–59 ml/min/1.73 m^2), median FGF23 (IQR) was 122 [90, 171]Stage 3b (GFR = 30–44 ml/min/1.73 m^2), median FGF23 (IQR) was 153 [102, 221] Stage 4 (GFR = 15–29 ml/min/1.73 m^2), median FGF23 (IQR) was 223 [148, 460]			
Gamma-glutamyl transferase (GGT, U/L) [1]	0 to <15 days	23–219	23–219
	15 days to <1 year	8–127	8–127
	1 to <11 years	6–16	6–16
	11 to <19 years	7–21	7–21
Homocysteine, total (tHcy, μmol/L) [1]	5 days to <1 year	2.9–10.0	2.9–10.0
	1 to <7 years	2.8–7.6	2.8–7.6
	7 to <12 years	3.4–8.5	3.4–8.5
	12 to <15 years	4.7–10.4	4.1–10.4
	15 to <19 years	5.5–13.4	4.9–11.9
Lactate dehydrogenase (LDH, U/L) [1]	0 to <15 days	309–1222	309–1222
	15 days to <1 year	163–452	163–452
	1 to <10 years	192–321	192–321
	10 to <15 years	170–283	157–272
	15 to <19 years	130–250	130–250
Lipase (U/L) [1]	0 to <19 years	4–39	4–39
Oxalate (μmol/L) [6]	3–12 months	1.56–5.58	1.56–5.58
	1–18 years	0.95–5.74	0.95–5.74
Protein electrophoresis, total protein (g/dL) [7] [**]To convert to SI units (g/L), multiply by 10	15 days to <1 year	5.1–7.3	5.1–7.3
	1–2 years	5.6–7.5	5.6–7.5
	3 to <16 years	6.0–8.0	6.0–8.0
	≥16 years	6.4–8.3	6.4–8.3
Retinol-binding protein (μmol/L) [8]	13–68 months	1.121 ± 0.22	1.121 ± 0.22
Xanthine (μmol/L) [9]	6–8 years	0.53 ± 0.2	0.53 ± 0.15

[*] Normal ranges values are given as International System (SI) units. For conversion to Conventional (CON) units, please refer to online conversion resources [10]

[**] Conventional units are reported as reference ranges, given their most frequent clinical use. Conversion factors to SI units are included for each analyte

Hematology[*]

	Age range	Male	Female
Activated partial thromboplastin time (sec) [11]	15 days to 4 weeks	27.6–45.6	27.6–45.6
	1–5 months	24.8–40.7	24.8–40.7
	6–11 months	25.1–40.7	25.1–40.7
	1–5 years	24.0–39.2	24.0–39.2
	6–10 years	26.9–38.7	26.9–38.7
	11–17 years	24.6–38.4	24.6–38.4
Antithrombin III (%) [12]	1–6 months	81–126	81–126
	7–12 months	90–132	90–132
	1–5 years	93–128	93–128
	6–10 years	92–122	92–122
	11–18 years	90–119	90–119
D-Dimer (ng/mL) [11]	15 days to 4 weeks	445–1200	445–1200
	1–5 months	90–878	90–878
	6–11 months	133–844	133–844
	1–5 years	88–780	88–780
	6–10 years	60–567	60–567
	11–16 years	69–580	69–580
Erythrocytes count (RBC count, x10^6/mcL) [7]	3–5 years	4.0–5.1	4.0–5.1
	6–10 years	4.1–5.2	4.1–5.2
	11–14 years	4.2–5.3	4.1–5.1
	>15 years	4.3–5.7	3.8–5.0
Erythrocyte sedimentation rate (mm/h) [13]	0–1 month	0–2	0–2
	1 month to 12 years	0–20	0–20
	>12 years	0–15	0–20
Erythropoietin (mIU/mL) [14, 15] [**]To convert to SI units (IU/L), multiply by 1	3 months to 1 year	0.8–24	0.8–24
	1–2 years	4.6–24.3	3.3–24.1
	3–5 years	4.2–20	3.5–15.9
	6–8 years	4.4–15.2	4.4–14.6
	9–11 years	4.3–15.9	3.8–14.4
Ferritin (pmol/L) [16, 1]	4 to <15 days	224–1611	224–1611
	15 days to <6 months	31–1454	31–1454
	6 months to <1 year	19–409	19–409
	1 to <5 years	12–224	12–224
	5 to <14 years	31–177	31–177
	14 to <16 years	28–186	12–152
	16 to <19 years	25–386	12–152
	CKD-5D[***]	>100.0	
Fibrinogen (g/L) [11]	15 days to 4 weeks	1.43–4.02	1.43–4.02
	1–5 months	1.50–3.76	1.50–3.76
	6–11 months	1.57–3.60	1.57–3.60
	1–5 years	1.88–4.13	1.88–4.13
	6–10 years	1.89–4.75	1.89–4.75
	11–17 years	1.77–4.20	1.77–4.20
Haptoglobin (g/L) [1]	0–15 days	0.00–0.10	0.00–0.10
	15 days to <1 year	0.07–2.21	0.07–2.21
	1 to <12 years	0.07–1.63	0.07–1.63
	12 to <19 years	0.07–1.79	0.07–1.79

(continued)

	Age range	Male	Female
Hemoglobin (g/dL) [7, 16] [**]To convert to SI units (g/L): multiply by 10	3–5 years	11.4–14.3	11.4–14.3
	6–8 years	11.5–14.3	11.5–14.3
	9–10 years	11.8–14.7	11.8–14.7
	11–14 years	12.4–15.7	11.9–14.8
	15–19 years	13.3–16.9	11.9–14.8
	>19 years	13.6–16.9	11.9–14.8
	CKD-5D	11.0–12.0	
Hematocrit (%) [7] [**]To convert to SI units (L/L): multiply by 0.01	3–5 years	34–42	34–42
	6–7 years	34–42	34–42
	8–11 years	35–43	35–43
	12–15 years	38–47	35–43
	>16 years	40–50	35–43
Iron (μmol/lt) [1]	0 to <14 years	2.8–22.9	2.8–22.9
	14 to <19 years	5.5–30.0	3.5–29.0
Protein C (%) [12]	1–6 months	41–115	41–115
	7–12 months	60–117	60–117
	1–5 years	63–133	63–133
	6–10 years	62–134	62–134
	11–18 years	71–144	71–144
Protein S (%) [12]	1–6 months	60–103	60–103
	7–12 months	61–95	61–95
	1–5 years	65–99	65–99
	6–10 years	63–97	63–97
	11–18 years	69–119	69–119
Prothrombin time (sec) [11]	15 days to 4 weeks	9.5–12.6	9.5–12.6
	1–5 months	9.7–12.8	9.7–12.8
	6–11 months	9.8–13.0	9.8–13.0
	1–5 years	9.9–13.4	9.9–13.4
	6–10 years	10.0–14.6	10.0–14.6
	11–17 years	10.0–14.1	10.0–14.1
Reticulocyte count (x10^9/L) [17]	8 months to 18 years	45–110	36–100
Reticulocyte hemoglobin content (CHr, pg) [18]	1–5 years	30.0–30.5	30.3–30.8
	6–11 years	31.1–31.6	31.4–31.7
	12–16 years	31.3–31.8	31.3–31.8
Transferrin (g/L) [1]	0 to <9 weeks	1.04–2.24	1.04–2.24
	9 weeks to <1 year	1.07–3.24	1.07–3.24
	1 to <19 years	2.20–3.37	2.20–3.37
	CKD-5D[***]	>20.0	
Transferrin saturation (TSAT, %) [16, 19]	0 to <1 year	4.1–59	4.1–59
	1 to <14 years	6.5–39	6.5–39
	14 to <19 years	9.6–58	5.2–44

[*] Normal ranges values are given as International System (SI) units. For conversion to Conventional (CON) units, please refer to online conversion resources [10]

[**] Conventional units are reported as reference ranges, given their most frequent clinical use. Conversion factors to SI units are included for each analyte

[***] CKD-5D: chronic kidney disease stage 5 with renal replacement therapy [16]

Hormones and Nutritional Parameters*

	Age range	Male	Female
Albumin G (bromcresol green, g/L) [1]	15 days to <1 year	28–47	28–47
	1 to <8 years	38–47	38–47
	8 to <15 years	41–48	41–48
	15 to <19 years	41–51	40–49
Aldosterone (pmol/L) [20]	<1 year	≤2868.8	≤3091.3
	1–5 years	≤1034.8	≤1734.8
	5–10 years	≤849.2	≤840.6
	10–15 years	≤678.1	≤828.9
	15–20 years	≤676.7	≤1086.5
Androgen Index, Free (FAI)(%) [1]	0–1 <year	0–32.7	0.0–1.3
	1 to <9 years	0.0–0.5	0.0–1.3
	9 to <14 years	0.2–34.7	1.1–2.6
	14 to <19 years	3.683.3	0.6–6.5
Antidiuretic hormone (pg/mL) [21, 22] **To convert to SI units (ng/L): multiply by 1	6–12 years	2.0–5.0	2.0–5.0
	13–18 years	1.7–3.5	1.7–3.5
Apolipoprotein AI (Apo AI) (g/L) [1]	15 days to <1 year	0.53–1.75	0.53–1.75
	1 to <14 years	0.8–1.64	0.8–1.64
	14 to <19 years	0.72–1.54	0.72–1.54
Apolipoprotein B (Apo B) (g/L) [1]	15 days to <1 year	0.19–1.23	0.19–1.23
	1 to <6 years	0.41–0.93	0.41–0.93
	6 to <19 years	0.31–0.84	0.31–0.84
Carnitine, free (μmol/L) [23]	0 to <1 year	11–53	11–53
	1 to <12 years	24–50	24–50
	12 to <19 years	25–52	18–44
Carnitine, total (μmol/L) [23]	0–1 year	23–77	23–77
	1–12 years	28–61	28–61
	12–19 years	32–58	25–52
Cholesterol, total (mmol/L) [1]	15 days to 1 year	1.68–6.67	1.68–6.67
	1–19 years	3.05–5.85	3.05–5.85
Copeptin (pmol/L) [24]	All pediatric ages	2.4–8.6	2.4–8.6
Copeptin [25]: Plasma copeptin levels after water deprivation test may be divided in: <2.2 pmol/L: suggest complete central diabetes insipidus 2.2–5 pmol/L: overlapping levels between complete diabetes insipidus and primary polydipsia (with 75% probability of complete diabetes insipidus if value is below 3.5 pmol/L and 25% probability if value is >3.5 pmol/L) >5–20 pmol/L: suggest primary polydipsia >20 pmol/L: suggest nephrogenic diabetes insipidus			
Cortisol (nmol/L) [20]	<1 year	66.6–547.3	59.7–439.6
	1–5 years	94.7–522.7	78.3–662.0
	5–10 years	100.2–422.4	89.6–427.7
	10–15 years	83.6–453.3	95.6–501.7
	15–20 years	113.9–591.6	135.7–1273.9
Dehydroepiandrosterone sulfate (DHEA-S) (μmol/L) [1]	0 to <2 months	28.9 to >40.7	28.9 to >40.7
	2 to <6 months	0.7–15.6	0.7–15.6
	6 months to <2 years	0.2–4.8	0.24.8
	1 to <6 years	0.1–3.0	0.1–3.0
	6 to <9 years	0.1–4.1	0.1–4.1

(continued)

	Age range	Male	Female
	9 to <13 years	0.9–7.3	0.9–7.3
	13 to <16 years	1.5–12.5	1.512.5
	16 to <19 years	3.4–18.2	4.0–15.5
Estradiol (pmol/L) [1]	15 days to <1 year	NA – 37	NA – 92
	1 to <9 years	NA – 176	NA – 46
	Tanner I	NA – 74	NA – 68
	Tanner II	NA – 96	NA – 67
	Tanner III	NA – 317	NA – 76
	Tanner IV	49 – 517	NA – 128
	Tanner V	69 – 762	64 – 126
Folate (nmol/L) [1]	0 to <14 years	27.6 to >56.2	27.6 to >56.2
	14 to <19 years	20.1 to >56.2	20.1 to >56.2
Follicle stimulating hormone (IU/L) (FSH) [1]	30 days–<1 year	0.1–2.4	0.4–10.4
	1 to <9 years	NA – 0.9	0.4–5.5 IU
	Tanner I	NA – 1.52	0.63–4.05
	Tanner II	NA – 2.98	0.27–5.76
	Tanner III	NA – 6.24	0.10–7.19
	Tanner IV	0.58 – 5.05	0.30–6.95
	Tanner V	0.79 – 7.19	0.41–8.59
High-density lipoprotein cholesterol (HDL-C) (mmol/L) [1]	15 days to <1 year	0.28–1.78	0.28–1.78
	1 to <4 years	0.8–1.56	0.8–1.56
	4 to <13 years	0.88–1.81	0.88–1.81
	13 to <19 years	0.78–1.70	0.79–1.79
Insulin (pmol/L) [1]	<1 year	7–163	7–163
	1 to <6 years	9–279	9–279
	6 to <11 year	11–257	11–257
	11 to <19 year	18–492	18–492
Low-density lipoprotein cholesterol (LDL) (mmol/L) [26]	<20 years	<2.8	<2.8
Luteinizing hormone (LH) (IU/L) [1]	4 days to <3 months	0.2–3.8	NA – 2.4
	3 months to <1 year	NA – 2.9	NA – 1.2
	1 to <10 years	NA – 0.3	NA – 0.3
	Tanner I	NA – 1.2	NA – 0.1
	Tanner II	NA – 1.2	NA – 2.3
	Tanner III	NA – 2.3	NA – 7.4
	Tanner IV	NA – 4.9	0.3–6.7
	Tanner V	NA – 5.9	0.4–21.2
Non-HDL cholesterol (mmol/L) [26]	<20 years	<3.1	<3.1
Prealbumin (g/L) [1]	15 days to <1 year	0.05–0.24	0.05–0.24
	1 to <5 years	0.12–0.23	0.12–0.23
	5 to <13 years	0.14–0.26	0.14–0.26
	13 to <16 years	0.18–0.31	0.18–0.31
	16 to <19 years	0.20–0.35	0.17–0.33
Progesterone (nmol/L) [1]	4 days to <1 year	NA – 2	NA – 4
	1 to <10 years	NA – 1	NA – 1
	Tanner I	NA – 1	NA – 1
	Tanner II	NA – 1	0–1
	Tanner III	NA – 2	1–2
	Tanner IV	NA –1	1–2
	Tanner V	0–2	NA – 34

(continued)

	Age range	Male	Female
Prolactine (mIU/L) [1]	4 to <30 days	264–4468	264–4468
	30 days to <1 year	132–2388	132–2388
	1 to <19 years	88–484	88–484
Protein, total (g/L) [1]	15 days- to 1 year	43–68	43–68
	1 to <6 years	59–72	59–72
	6 to <9 years	62–74	62–74
	9 to <19 years	63–77	63–77
PTH (intact and biointact) (pg/ml) [1] **To convert to SI units (pmol/L): multiply by 0.1053	6 days to 1 year	6.4–88.6	6.4–88.6
	1–9 years	16.2–63	16.2–63
	9–17 years	21.9–87.6	21.9–87.6
	17–19 years	16.0–64	16.0–64
Renin (erect/recumbent) (pmol/ml/hr) [27]	0–3 months	4.0–12.0	4.0–12.0
	3 months to 5 years	2.0–6.0	2.0–6.0
	6–9 years	1.0–4.0	1.0–4.0
	10–16 years	0.6–2.2	0.6–2.2
Testosterone, calculated free (cFT) (pmol/L) [1]	0–1 year	0.1–198.4	0.3–9.1
	1 to <9 years	0.3–4.0	0.3–9.1
	9–14 years	1.5–250.6	1.0–16.4
	14–<19 years	17.4–494.0	4.9–34.3
Thyroid-stimulating hormone (mIU/L) [1]	4 days to <6 months	0.73–4.77	0.73–4.77
	6 months to <14 years	0.70–4.17	0.70–4.17
	14–19 years	0.47–3.41	0.47–3.41
Thyroxine, free (pmol/L) [1]	5–15 days	13.5–41.3	13.5–41.3
	15–29 days	8.7–32.5	8.7–32.5
	30 days to <1 year	11.4–21.9	11.4–21.9
	1–19 years	11.4–17.6	11.4–17.6
Triglycerides (mmol/L) [1]	15 days to 1 year	0.67–3.33	0.67–3.33
	1 to <9 years	0.55–2.54	0.55–2.54
	10–19 years	0.55–2.54	0.55–2.54
Uric acid (μmol/lt) [1]	15 days to 1 year	94–377	94–377
	1 to <12 years	106–289	106–289
	12 to <19 years	156–454	153–349
Vitamin A (μmol/lt) [1]	0 to <1 year	0–2	0–2
	1 to <11 years	1–2	1–2
	11 to<16 years	1–2	1–2
	16 to<19 years	1–3	1–3
Vitamin B6 (nmol/L) [28]	0 to >18 years	>30	>30
Vitamin B12 (pmol/L) [1]	0 to <1 year	118 to >1107	118 to >1107
	1 to <2 years	197 to >1107	197 to >1107
	2 to <8 years	190–747	190–747
	8 to <14 years	149–772	149–772
	14 to <19 years	132–531	132–531
Vitamin C (μmol/lt) [29]	0 to >18 years	>11.4	>11.4
Zinc (μmol/L) [30]	6 months to 2 years	8.6–19.1	8.6–19.1
	3–4 years	9.2–18.4	9.2–18.4
	5–6 years	9.8–17.9	9.8–17.9
	7–8 years	9.9–19.1	9.9–19.1
	9–10 years	10.1–19.1	10.1–19.1

(continued)

	Age range	Male	Female
	11–12 years	10.1–19.4	10.1–19.4
	13–14 years	10.6–19.0	10.6–19.0
	15–16 years	9.5–18.8	9.5–18.8
	17–18 years	9.5–20.3	9.5–20.3
1,25-dihydroxyvitamin D (pg/mL) [31] [**]To convert to SI units (pmol/L): multiply by 2.4038	<1 year	30–181	30–181
	1 to <3 year	43–140	43–140
	3 to <19 years	42–95	42–95
25-hydroxyvitamin D (nmol/L) [1, 32]	0 to <1 year	27.5–159	27.5–159
	1 to <12 years	43.4–121	43.4–121
	12 to <19 years	29.0–105	29.0–105
	CKD-5D[***]	>74.8	>74.8

[*] Normal ranges values are given as International System (SI) units. For conversion to Conventional (CON) units, please refer to online conversion resources [10]

[**] Conventional units are reported as reference ranges, given their most frequent clinical use. Conversion factors to SI units are included for each analyte

[***] CKD-5D: chronic kidney disease stage 5 with renal replacement therapy

Markers of Kidney Function[*]

	Age range	Male	Female
Blood urea nitrogen (mg/dL) [1] [**]To convert to SI units (mmol/L): multiply by 0.357	0 to <15 days	2.8–23	2.8–23
	15 days to <1 year	3.4–16.8	3.4–16.8
	1 to <10 years	9.0–22.1	9.0–22.1
	10 to <19 years	7.3–21.0	7.3–19.0
Creatinine, enzymatic (mg/dL) [1] [**]To convert to SI units (μmol/L): multiply by 88.42	0 to <15 days	0.32–0.92	0.32–0.92
	15 days to <2 years	0.10–0.36	0.10–0.36
	2 years to <5 years	0.20–0.43	0.20–0.43
	5 years to <12 years	0.31–0.61	0.31–0.61
	12 to <15 years	0.45–0.81	0.45–0.81
	15 to <19 years	0.62–1.08	0.49–0.84
Creatinine, Jaffe (mg/dL) [1] [**]To convert to SI units (μmol/L): multiply by 88.42	0 to <15 days	0.42–1.05	0.42–1.05
	15 days to <1 year	0.31–0.53	0.31–0.53
	1 to <4 years	0.39–0.55	0.39–0.55
	4 to <7 years	0.44–0.65	0.44–0.65
	7 to <12 years	0.52–0.69	0.52–0.69
	12 to <15 years	0.57–0.80	0.57–0.80
	15 to <17 years	0.651.04	0.59–0.86
	17 to <19 years	0.69–1.10	0.60–0.88

Formulas for GFR assessment:

A. Creatinine-based estimated GFR (eGFR) equations

- ***Schwartz equation*:**

  Using Jaffe assay (1976) [33, 34]: eGFR = k × height/Scr

  (*where height is in cm, Scr in mg/dL, and k depends on age: k = 0.33 for preterm infants throughout the first year of life; k = 0.45 for term infants throughout the first year of life; k = 0.55 for children and adolescent girls; k = 0.7 for adolescent boys* (*≥13 years old*))

  Using enzymatic assay (2009) [35]: eGFR = 0.413 × height/Scr

  (*where height is in cm, Scr in mg/dL*)

(continued)

- ***Pottel eq.*** (2012) [36]:
  $\text{eGFR} = 107.3/(\text{Scr}/Q)$
  (*where Q is the median Scr for healthy children of a particular age*)
- Gao quadratic formula (2013) [37]:
  Females: $0.68 \times (\text{Height}/\text{Scr}) - 0.0008 \times (\text{height}/\text{Scr})^2 + 0.48 \times \text{age} - 21.53$
  Males: $0.68 \times (\text{Height}/\text{Scr}) - 0.0008 \times (\text{height}/\text{Scr})^2 + 0.48 \times \text{age} - 25.68$
  (*where height is in cm, Scr in mg/dl, age in years*)

B. Cystatine C-based eGFR equations

- ***Zappitelli*** (2006) [38]:
  $\text{eGFR} = 75.94 \times \text{CysC}^{-1.17}$ if renal transplant, × 1.2
- ***Schwartz eq.*** (2012) [39, 40]:
  $\text{eGFR} = 70.69 \times (\text{CysC}^{-0.931})$
  (*If using a cystatin C platform based on the ERM_DA471/IFCC reference material, dividing IFCC-calibrated cystatin C levels by 1.17 and using this value in the equations listed above has been suggested*)

C. Combined creatinine and cystatin C equations

- ***Zappitelli*** (2006) [38]:
  $\text{eGFR} = (43.82 \times \exp.(0.003 \times \text{height}))/(\text{CysC}^{0.635} \times \text{Scr}^{0.547}) \times 1.165$ if renal transplant and $\times 1.57\text{Scr}^{0.925}$ if spina bifida (*where Scr is in mg/dl*)
- ***Schwartz*** (***CKiD***, 2009) [41]**:**
  $\text{eGFR} = 39{,}1 \times (\text{talla}/\text{Scr})^{0{,}516} \times (1{,}8/\text{CysC})^{0{,}294} \times (30/\text{BUN})^{0{,}169} \times 1{,}099^{\text{varón}} \times (\text{height}/1{,}4)^{0{,}188}$
  (*where height is in m, Scr in mg/dl, CysC in mg/l, and BUN in mg/dl*)
- ***Schwartz***, (***CKiD***, 2012) [39, 40]:
  $\text{eGFR} = 39.8 \times [\text{height}/\text{Scr}]^{0.456} \times [1.8/\text{Cys C}]^{0.418} \times [30/\text{BUN}]^{0.079} \times [1.076]^{\text{male}} \times [\text{height}/1.4]^{0.179}$
  (*where height is in m, Scr in mg/dl, Cys C in mg/L, and BUN in mg/dl. If using a cystatin C platform based on the ERM_DA471/IFCC reference material, dividing IFCC-calibrated cystatin C levels by 1.17 and using this value in the equations listed above has been suggested*)
- ***Chehade*** (2014) [42]:
  Female patients: $0.42 \times (\text{Ht}/\text{SCreat}) - 0.04 \times (\text{Ht}/\text{SCreat})^2 - 14.5 \times \text{CysC} + 0.69 \times \text{age} + 18.25$
  Male patients: $0.42 \times (\text{Ht}/\text{SCreat}) - 0.04 \times (\text{Ht}/\text{SCreat})^2 - 14.5 \times \text{CysC} + 0.69 \times \text{age} + 21.88$
  (*where Ht is in cm, SCreat in mg/dl, CysC in mg/L, and age in years*)

Glomerular filtration rate (mL/min/1.73 m^2) (GFR) [43]	**Preterm**		
	7 days	19.9 ± 9.3	19.9 ± 9.3
	14 days	22.1 ± 14.9	22.1 ± 14.9
	21 days	24.6 ± 10.8	24.6 ± 10.8
	28 days	27.8 ± 12.6	27.8 ± 12.6
	Full term		
	0–3 months	60.4 ± 17.4	60.4 ± 17.4
	4–6 months	87.4 ± 22.3	87.4 ± 22.3
	7–12 months	96.2 ± 12.2	96.2 ± 12.2
	1–2 years	105.2 ± 17.3	105.2 ± 17.3
	Children		
	3–4 years	111.2 ± 18.5	111.2 ± 18.5
	5–6 years	114.1 ± 18.6	114.1 ± 18.6
	7–8 years	111.3 ± 18.3	111.3 ± 18.3
	9–10 years	110.0 ± 21.6	110.0 ± 21.6
	11–12 years	116.4 ± 18.9	116.4 ± 18.9
	13–15 years	117.2 ± 16.1	117.2 ± 16.1
GFR estimated by creatinine clearance in preterm newborn and inulin clearance in full-term newborn and children			

[*] Normal ranges values are given as International System (SI) units. For conversion to Conventional (CON) units, please refer to online conversion resources [10]

[**] Conventional units are reported as reference ranges, given their most frequent clinical use. Conversion factors to SI units are included for each analyte

Neonatology*

	Age range	Male	Female
Albumin (g/L) [44]	1–12 h	28–43	28–43
	12–24 h	28–43	28–43
	24–48 h	28–43	28–43
	48–72 h	28–43	28–43
Aldosterone (erect/recumb) (pmol/L) [27] 1 pmol = 0.001 nmol	0–3 months	1000–3500	1000–3500
Alkaline phosphatase (U/L) [1]	1–72 h	28–300	28–300
Alpha fetoprotein (ug/L) [1] SI units not provided	month 1	>2000	>2000
Amylase (U/L) [1]	0–15 days	3–10	44,107
Apolipoprotein B (g/L) [1]	0–15 days	0.09–0.67	0.09–0.67
Aspartate aminotransferase U/L [1]	0–15 days	32–162	32–162
Bilirubin, direct (uMol/L) [1]	0–14 days	5.7–12.1	5.7–12.1
Bilirubin, total (uMol/L) [1]	0–15 days	3.3283	3.3–283
Calcium, ionized (mmol/L) [44]	1–12 h	1.05–1.37	1.05–1.37
	12–24 h	1.05–1.37	1.05–1.37
	24–48 h	1.05–1.37	1.05–1.37
	48–72 h	1.10–1.44	1.10–1.44
Calcium, total (mmol/L) [44]	1–12 h	1.8–2.3	1.8–2.3
	12–24 h	1.7–2.4	1.7–2.4
	24–48 h	1.5–2.5	1.5–2.5
	48–72 h	1.5–2.4	1.5–2.4
Chloride (meq/L) [44] **To convert to SI units (mmol/L): multiply by 1	1–12 h	80–111	80–111
	12–24 h	87–114	87–114
	24–48 h	92–114	92–114
	48–72 h	93–112	93–112
Cholesterol (mmol/L) [1]	0–15 days	1.2–3.2	1.1–2.82
Cortisol (nmol/L) [44]	1–48 h	200–700	200–700
C-Peptide (pmol/L) [1] 1 pmol = 0.001 nmol	0–1 year	70–1448	70–1448
Hematocrit (%) [44] **To convert to SI units (L/L): multiply by 0.01	12–24 h	58	58
	48–72 h	55	55
Hemoglobin (g/L) [44]	12–24 h	184	184
	48–72 h	178	178
High-density lipoprotein cholesterol (mmol/L) [1]	0–15 days	0.4–1.08	0.4–1.08
Lactate (mmol/L) [44]	1–12 h	0.9–2.7	0.9–2.7
	12–24 h	0.8–1.2	0.8–1.2
Magnesium (mmol/L) [44, 1]	1–12 h	N/A	N/A
	12–24 h	0.72–1.00	0.72–1.00
	24–48 h	0.82–1.62	0.82–1.62
	48–72 h	0.81–1.05	0.81–1.05
Maximum osmolality (mOsm/L) [45]	2–4 days	346–559	346–559
	4–6 days	559–663	559–663
	6–8 days	663–728	663–728
	8–10 days	728–775	728–775
	10–30 days	775–963	775–963

(continued)

	Age range	Male	Female
Phosphate (mmol/L) [44, 1]	1–12 h	1.97 (1.1–2.8)	1.97 (1.1–2.8)
	12–24 h	1.84 (0.9–2.6)	1.84 (0.9–2.6)
	24–48 h	1.91 (1.0–2.8)	1.91 (1.0–2.8)
	48–72 h	1.87 (0.9–2.5)	1.87 (0.9–2.5)
	0–15 days	1.8–3.4	1.8–3.4
Potassium (mmol/L) [44]	1–12 h	6.4 (5.3–7.3)	6.4 (5.3–7.3)
	12–24 h	6.3 (5.3–8.9)	6.3 (5.3–8.9)
	24–48 h	6.0 (5.2–7.3)	6.0 (5.2–7.3)
	48–72 h	5.9 (5.0–7.7)	5.9 (5.0–7.7)
Sodium (mmol/L) [44]	1–12 h	143 (124–156)	143 (124–156)
	12–24 h	145 (132–159)	145 (132–159)
	24–48 h	148 (134–160	148 (134–160
	48–72 h	149 (139–162)	149 (139–162)
Total Protein (g/L) [1]	0–15 days	53–83	53–83
Triglycerides (mmol/L) [1]	0–15 days	0.98–3.21	0.98–3.21
Troponin I (ng/L) [1]	0–15 days	3–936	3–936
TSH (mIU/L) [1]	12–24 h	3.0–120	3.0–120
	24–48 h	3.0–30	3.0–30
Uric Acid (umol/L) [1]	0–15 days	164–757	164–757
Vitamin D (nmol/L) [1]	0–15 days	4–85	4–85
WCC × 10^9/L [44]	1–12 h	22.8 (13–38)	22.8 (13–38)
	12–24 h	18.9 (9.4–34)	18.9 (9.4–34)
17-OH Progesterone (nmol/L) [44]	48–72 h	0.7–12.4	0.7–12.4

[*] Normal ranges values are given as International System (SI) units. For conversion to Conventional (CON) units, please refer to online conversion resources [10]

[**] Conventional units are reported as reference ranges, given their most frequent clinical use. Conversion factors to SI units are included for each analyte

Renal Ultrasound Values

	Age range		Male		Female	
			Left	Right	Left	Right
Kidney size [46, 47]	6 months	Length (mm)	60.1(48.6–70.4)	58.8(49.7–69.3)	58.6(50.0–67.4)	57.5(49.5–67.6)
		Width (mm)	28.2(22.3–36.4)	28.0(21.8–35.4)	27.6(21.6–35.7)	27.5(21.4–36.5)
		Depth (mm)	26.5(20.1–33.0)	27.8(21.3–34.9)	25.5(20.2–32.2)	27.0(20.9–34.0)
		Volume (cm^3)	23.8(14.0–36.6)	24.0(15.3–35.6)	21.9(13.9–33.4)	22.4(14.2–34.6)
	24 months	Length (mm)	66.8(56.1–80.3)	65.2(54.8–76.6)	65.7(53.7–78.0)	64.3(53.2–76.5)
		Width (mm)	30.8(25.6–37.1)	30.7(25.6–38.0)	30.1(23.5–37.8)	30.6(25.2–37.1)
		Depth (mm)	30.9(24.8–38.9)	32.0(25.5–39.4)	30.1(24.2–38.1)	31.9(25.9–39.6)
		Volume (cm^3)	33.6(22.8–51.6)	33.8(22.9–53.3)	31.8(19.3–52.2)	33.1(22.1–49.2)
	2 years	Length (cm)	6.98(6.68–7.29)	6.76(6.47–7.04)	7.17(6.48–7.86)	6.78(5.78–7.78)
		Volume (cm^3)	48.47(42.31–54.63)	45.35(40.94–49.74)	52.14(37.93–66.34)	44.16(27.78–60.54)
	3 years	Length (cm)	7.11(6.66–7.56)	6.91(6.49–7.33)	7.54(7.08–8.00)	7.29(6.77–7.81)
		Volume (cm^3)	51.89(42.60–61.17)	46.47(40.92–52.02)	58.13 (52.18–64.07)	51.56(40.32–62.80)
	4 years	Length (cm)	7.46(7.15–7.76)	7.19(6.81–7.57)	7.58(7.18–7.98)	7.37(6.91–7.82)
		Volume (cm^3)	60.48(50.37–70.60)	58.71(49.45–67.96)	64.05(55.46–72.64)	55.07(47.00–63.14)

(continued)

	Age range		Male		Female	
			Left	Right	Left	Right
	5 years	Length (cm)	7.94(7.47–8.41)	7.93(7.39–8.46)	7.96(7.58–8.34)	7.38(7.09–7.67)
		Volume (cm^3)	73.25(64.75–81.76)	63.64(56.11–71.17)	73.60(61.75–85.44)	63.91 (53.99–73.82)
	6 years	Length (cm)	7.82(7.38–8.25)	7.49(7.10–7.88)	7.78(7.27–8.29)	7.66(7.15–8.17)
		Volume (cm^3)	68.26(59.22–77.31)	61.91(55.04–68.78)	68.82(53.75–83.89)	66.84(54.34–79.35)
	7 years	Length (cm)	8.84(8.23–9.46)	8.46(7.75–9.17)	8.15(7.68–8.62)	7.98(7.40–8.56)
		Volume (cm^3)	85.44(75.76–95.13)	76.57 (66.07–87.07)	76.59(59.16–94.03)	70.20(56.34–84.05)
	8 years	Length (cm)	8.68(8.08–9.27)	8.39(7.91–8.87)	8.73(8.23–9.23)	8.33(7.82–8.84)
		Volume (cm^3)	92.22 (80.73–103.71)	87.45 (73.93–100.97)	89.72(79.69–99.75)	77.59(72.63–82.56)
	9 years	Length (cm)	8.92(8.47–9.38)	8.69(8.13–9.24)	9.04(8.73–9.36)	8.51(8.14–8.88)
		Volume (cm^3)	101.84 (83.77–119.91)	93.11(80.26–105.96)	92.87 (82.18–103.55)	85.36(74.61–96.12)
	10 years	Length (cm)	8.89(8.39–9.38)	8.77(8.47–9.07)	9.10(8.38–9.81)	8.82(8.17–9.47)
		Volume (cm^3)	109.80(95.84–123.75)	98.82(86.95–110.69)	105.00(84.58–125.42)	94.77(77.83–111.70)
	11 years	Length (cm)	8.88(8.38–9.38)	8.55(7.97–9.12)	9.31(8.81–9.82)	8.98(8.52–9.44)
		Volume (cm^3)	116.37(76.42–156.32)	104.39(70.62–138.15)	108.96(93.44–124.48)	99.48(86.64–112.32)
	12 years	Length (cm)	9.61(9.20–10.03)	9.20(8.61–9.79)	10.42 (9.83–11.00)	9.68(8.79–10.57)
		Volume (cm^3)	142.07 (122.77–161.37)	131.22(104.60–157.84)	143.02(127.47–158.58)	127.88 (108.88–146.89)
	13–18 years	Length (cm)	10.60(10.28–10.91)	10.25(9.96–10.54)	10.49(10.22–10.76)	9.96(9.70–10.22)
		Volume (cm^3)	166.22(153.39–179.06)	149.87 (140.32–159.42)	143.36 (133.60–153.13)	128.21 (120.48–135.94)
	Height		**Mean kidney length (cm)**			
	85–95cm	Right	6.81 (6.62–7.00)			
		Left	7.07 (6.86–7.28)			
	96–105cm	Right	7.38 (7.09–7.68)			
		Left	7.65 (7.36–7.95)			
	106–115 cm	Right	7.41 (7.15–7.66)			
		Left	7.63 (7.41–7.86)			
	116–125 cm	Right	7.72 (7.47–7.98)			
		Left	7.99 (7.75–8.24)			
	126–135 cm	Right	8.44 (8.15–8.73)			
		Left	8.71 (8.42–8.99)			
	136–145 cm	Right	8.51 (8.31–8.71)			
		Left	8.81 (8.60–9.01)			
	146–155 cm	Right	9.26 (8.98–9.54)			
		Left	9.67 (9.37–9.97)			
	156–165 cm	Right	9.87 (9.59–10.15)			
		Left	10.47 (10.22–10.72)			
	166–175 cm	Right	10.05 (9.76–10.35)			
		Left	10.53 (10.18–10.88)			
	176–185 cm	Right	10.63 (10.23–11.03)			
		Left	10.51 (9.65–11.36)			

Rheumatology*

	Age range	Male	Female
ANA (indirect IF with HEp-2) [48, 49]	6 months to 5 years	<1:80	<1:80
	5–12 years	<1:80	<1:80
	12–19 years	<1:80	<1:80
Anti-dsDNA (INOVA ELISA, IU/mL) [50, 51]	6–16 years	<30	<30
Antistreptolysin O (ASO) (IU/mL) [1]	0 to <6 months	0	0
	6 months to <1 years	0–30	0–30
	1 to <6 years	0–104	0–104
	6 to <19 years	0–331	0–331
c-ANCA [52]	5–17 years	<1:40	<1:40
CH50 (hemolytic method, units/mL) [53]	0–14 years	73–213	73–213
Complement C3 (mg/dL) [1] SI units not provided	0 to <15 days	50–121	50–121
	15 days to 1 years	51–160	51–160
	1 to <19 years	83–152	83–152
Complement C4 (mg/dL) [54] SI units not provided	0–2 years	11.3–39.1	11.3–39.1
	3–4 years	11.0–41.9	11.0–41.9
	5–9 years	7.3–33.7	7.3–33.7
	10–14 years	11.4–36.7	11.4–36.7
	15–18 years	10.3–46.9	10.3–46.9
C-reactive protein – high sensitivity (CRP) (mg/L) [1]	0 to <15 days	0.3–5.8	0.3–5.8
	15 days to <15 years	0.1–0.9	0.1–0.9
	15 to <19 years	0.1–1.6	0.1–1.6
C1q (μg/mL) [55] SI units not provided	1–2 years	39–151	53–165
	3–4 years	73–137	81–145
	5–6 years	75–143	84–152
	7–8 years	55–183	60–188
	9–10 years	94–146	93–145
	11–13 years	68–144	76–152
	14–16 years	65–185	79–199
	17–19 years	90–182	99–191
Factor B (μg/mL) [55, 56] SI units not provided	1–2 years	19–55	13–61
	3–4 years	20.6–58.8	20.6–58.8
	5–6 years	25.6–60.1	25.6–60.1
	7–9 years	20.9–53.2	20.9–53.2
	10–14 years	18.5–51.3	18.5–51.3
Immunoglobulin A (mg/dL) [1] **To convert to SI units (g/L): multiply by 0.01	0 to <1 year	1.0–29	1.0–29
	1 to <3 years	4.0–90	4.0–90
	3 to <6 years	26–147	26–147
	6 to <14 years	47–221	47–221
	14 to <19 years	53–287	53–287
Immunoglobulin G (mg/dL) [1] **To convert to SI units (g/L): multiply by 0.01	0 to <15 days	320–1407	320–1407
	15 days to <1 year	108–702	108–702
	1 to <4 years	316–1148	316–1148
	4 to <10 years	542–1358	542–1358
	10 to <19 years	658–1534	658–1534
Immunoglobulin M (mg/dL) [1] **To convert to SI units (g/L): multiply by 0.01	15 days to <13 weeks	12.0–71	12.0–71
	13 weeks to <1 year	16–86	16–86
	1 to <19 years	39–151	39–151

(continued)

	Age range	Male	Female
p-ANCA [52]	5–17 years	<1:40	<1:40
Rheumatoid factor (IU/mL) [1, 57]	0 to <15 days	9.0–17.1	9.0–17.1
	1–15 years	≤15	≤15

*Normal ranges values are given as International System (SI) units. For conversion to Conventional (CON) units, please refer to online conversion resources [10]

**Conventional units are reported as reference ranges, given their most frequent clinical use. Conversion factors to SI units are included for each analyte

Serum Electrolytes and Blood Gases*

	Age range	Male	Female
Arterial blood pH [58]	>1 day to >18 years	7.35–7.45	7.35–7.45
Bicarbonate (mmol/L) [59, 60]	1 month to <2 years	20–22	20–22
	2–18 years	22–24	22–24
Calcium ionized (mmol/L) [32]	0–5 months	1.22–1.40	1.22–1.40
	6–12 months	1.2–1.40	1.2–1.40
	1–5 years	1.22.32	1.22–1.32
	6–12 years	1.15–1.32	1.15–1.32
	13–20 years	1.12–1.30	1.12–1.30
Carbon dioxide (mmol/L) [1]	0–14 days	5–20	5–20
	15 days to <1 year	10–24	10–24
	1 to <5 years	14–24	14–24
	5 to <15 years	17–26	17–26
	15 to <19 years	18–28	17–26
Chloride (mmol/L) [2]	0–7 days	97–108	97–108
	7–31 days	97–108	97–108
	1–6 months	97–108	97–108
	6 months to 1 year	97–106	97–106
	>1 year	97–107	97–107
Magnesium (mmol/L) [1]	15 days to 1 year	0.81–1.27	0.81–1.27
	1 to <19 years	0.86–1.17	0.86–1.17
Osmolality (mmol/Kg) [58]	0–16 years	271–296	271–296
Phosphate (mmol/L) [1]	0 to <15 days	1.80–3.40	1.80–3.40
	15 days to <1 year	1.54–2.72	1.54–2.72
	1 to <5 years	1.38–2.19	1.38–2.19
	5 to <13 years	1.33–2.19	1.33–2.19
	13 to <16 years	1.14–1.99	1.02–1.79
	16 to <19 years	0.95–1.62	0.95–1.62
Potassium (mmol/L) [2]	0–7 days	3.2–5.7	3.2–5.7
	7–31 days	3.4–6.2	3.4–6.2
	1–6 months	3.5–5.8	3.5–5.8
	6 months to 1 year	3.5–6.3	3.5–6.3
	>1 year	3.3–4.7	3.3–4.7

(continued)

	Age range	Male	Female
Sodium (mmol/L) [2]	0–7 days	131–144	131–144
	7–31 days	132–142	132–142
	1–6 months	132–140	132–140
	6 months to 1 year	131–140	131–140
	>1 year	132–140	132–140
Total calcium (mmol/L) [1]	0 to <1 year	2.13–2.74	2.13–2.74
	1 to <19 years	2.29–2.63	2.29–2.63

*Normal ranges values are given as International System (SI) units. For conversion to Conventional (CON) units, please refer to online conversion resources [10]

Urine Studies and Tubular Function*

Urinary anion gap	Age range	Male	Female
Anion gap (mmol/L) [61, 62, 63]	Neonates (not reliable)	N/A	N/A
	Infants-adult	without acidosis: 23–95	without acidosis: 23–95
		with acidosis: −67 to −5	with acidosis: −67 to −5
Urinary acute kidney injury biomarkers			
IL-18 (pg/ml) [64]	3 to <5 years	<78.3	<100.5
	5 to <10 years	<41.7	<79.0
	10 to <15 years	<58.5	<111.1
	15 to <18 years	<71.2	<273.1
KIM-1 (ng/ml) [64]	3 to <5 years	<983.7	<1291.7
	5 to <10 years	<1276.6	<1212.9
	10 to <15 years	<1156.6	<1103.5
	15 to <18 years	<1876.2	<1934.5
LFABP (ng/ml) [64]	3 to <5 years	<49.4	<41.4
	5 to <10 years	<15.5	<13.5
	10 to <15 years	<13.7	<13.3
	15 to <18 years	<10.5	<6.7
NGAL (ng/ml) [64]	3 to <5 years	<26.1	<52.2
	5 to <10 years	<10.9	<139.5
	10 to <15 years	<22.5	<72.3
	15 to <18 years	<50	<138.6
NGAL [65]: cutoff values to predict AKI have generally ranged from 100–270 ng/mL. In children, a pooled analysis showed a cutoff of 135 ng/mL has shown a 77.6% sensitivity and 88.0% sensitivity to predict AKI			
TIMP2-IGBP7 (ng/ml)^2/1000 ([66])	0–28 days	0.08–0.27	0.08–0.27
	29 days to 2 years	0.02–0.25	0.02–0.25
	2–5 years	0.20–0.84	0.20–0.84
	6–11 years	0.26–1.07	0.26–1.07
	12–18 years	0.07–0.46	0.07–0.46
TIMP2-IGBP7 [67]: cutoff values to predict AKI are as follows: ≤0.3, low risk; >0.3 and ≤2.0, moderate risk; and >2.0, high risk			

(continued)

Urinary anion gap	Age range	Male	Female
Urinary amino acids [68]			
Alpha-amino butyric acid (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	<80	<80
	1–6 months	<62	<62
	6–12 months	<71	<71
	1–2 years	<71	<71
	2–4 years	<53	<53
	4–7 years	<44	<44
	7–10 years	<44	<44
	10–13 years	<44	<44
	>13 years	<35	<35
Alanine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	663–2157	663–2157
	1–6 months	636–1821	636–1821
	6–12 months	318–1432	318–1432
	1–2 years	362–1149	362–1149
	2–4 years	292–1017	292–1017
	4–7 years	239–813	239–813
	7–10 years	150–575	150–575
	10–13 years	186–548	186–548
	>13 years	141–601	141–601
Arginine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	<124	<124
	1–6 months	<97	<97
	6–12 months	<97	<97
	1–2 years	<71	<71
	2–4 years	<80	<80
	4–7 years	<62	<62
	7–10 years	<53	<53
	10–13 years	<53	<53
	>13 years	<44	<44
Asparagine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	<743	<743
	1–6 months	<513	<513
	6–12 months	<318	<318
	1–2 years	<283	<283
	2–4 years	<265	<265
	4–7 years	<256	<256
	7–10 years	<212	<212
	10–13 years	<159	<159
	>13 years	<203	<203
Aspartic acid (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	18–106	18–106
	1–6 months	18–141	18–141
	6–12 months	27–106	27–106
	1–2 years	27–88	27–88
	2–4 years	18–71	18–71
	4–7 years	18–71	18–71
	7–10 years	9–80	9–80
	10–13 years	9–88	9–88
	>13 years	18–62	18–62
Beta-amino isobutyric acid (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	<769	<769
	1–6 months	<1909	<1909
	6–12 months	<2351	<2351

(continued)

Urinary anion gap	Age range	Male	Female
	1–2 years	<1821	<1821
	2–4 years	<1547	<1547
	4–7 years	<522	<522
	7–10 years	<751	<751
	10–13 years	<513	<513
	>13 years	<804	<804
Citrulline (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	<97	<97
	1–6 months	<88	<88
	6–12 months	<71	<71
	1–2 years	<62	<62
	2–4 years	<53	<53
	4–7 years	<44	<44
	7–10 years	<44	<44
	10–13 years	<44	<44
	>13 years	<35	<35
½ Cystine (μmol/g creatinine) (reported as ½ Cystine because initial methods of quantification involved a reduction of cystine followed by an assay for cysteine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	212–690	212–690
	1–6 months	115–424	115–424
	6–12 months	106–256	106–256
	1–2 years	88–230	88–230
	2–4 years	71–265	71–265
	4–7 years	71–194	71–194
	7–10 years	71–186	71–186
	10–13 years	62–203	62–203
	>13 years	53–301	53–301
Glutamic acid (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	<265	<265
	1–6 months	<256	<256
	6–12 months	<159	<159
	1–2 years	<97	<97
	2–4 years	<88	<88
	4–7 years	<71	<71
	7–10 years	<44	<44
	10–13 years	<80	<80
	>13 years	<106	<106
Glutamine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	460–1812	460–1812
	1–6 months	557–2024	557–2024
	6–12 months	654–1742	654–1742
	1–2 years	548–1459	548–1459
	2–4 years	398–2086	398–2086
	4–7 years	460–1176	460–1176
	7–10 years	274–857	274–857
	10–13 years	177–990	177–990
	>13 years	177–672	177–672
Glycine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	2502–9698	2502–9698
	1–6 months	1856–6568	1856–6568
	6–12 months	1008–3934	1008–3934
	1–2 years	972–3147	972–3147
	2–4 years	981–2882	981–2882
	4–7 years	804–2175	804–2175
	7–10 years	743–2086	743–2086

(continued)

Urinary anion gap	Age range	Male	Female
	10–13 years	566–1459	566–1459
	>13 years	380–1529	380–1529
Histidine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	707–2608	707–2608
	1–6 months	636–3023	636–3023
	6–12 months	813–2458	813–2458
	1–2 years	769–2537	769–2537
	2–4 years	601–2254	601–2254
	4–7 years	539–1909	539–1909
	7–10 years	398–1627	398–1627
	10–13 years	380–1406	380–1406
	>13 years	230–1353	230–1353
Hydroxyproline (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	177–2829	177–2829
	1–6 months	<1264	<1264
	6–12 months	<194	<194
	1–2 years	<115	<115
	2–4 years	<115	<115
	4–7 years	<115	<115
	7–10 years	<115	<115
	10–13 years	<115	<115
	>13 years	<115	<115
Isoleucine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	<53	<53
	1–6 months	<44	<44
	6–12 months	<53	<53
	1–2 years	<53	<53
	2–4 years	<44	<44
	4–7 years	<44	<44
	7–10 years	<53	<53
	10–13 years	<53	<53
	>13 years	<35	<35
Leucine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	27–221	27–221
	1–6 months	35–106	35–106
	6–12 months	35–141	35–141
	1–2 years	27–150	27–150
	2–4 years	35–159	35–159
	4–7 years	27–115	27–115
	7–10 years	27–141	27–141
	10–13 years	27–124	27–124
	>13 years	18–97	18–97
Lysine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	194–1512	194–1512
	1–6 months	133–1759	133–1759
	6–12 months	115–698	115–698
	1–2 years	141–610	141–610
	2–4 years	88–407	88–407
	4–7 years	88–601	88–601
	7–10 years	88–389	88–389
	10–13 years	88–495	88–495
	>13 years	62–513	62–513

(continued)

Urinary anion gap	Age range	Male	Female
Methionine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	62–239	62–239
	1–6 months	53–194	53–194
	6–12 months	71–256	71–256
	1–2 years	62–256	62–256
	2–4 years	44–186	44–186
	4–7 years	44–177	44–177
	7–10 years	27–150	27–150
	10–13 years	27–88	27–88
	>13 years	18–141	18–141
3-Methylhistidine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	177–345	177–345
	1–6 months	168–354	168–354
	6–12 months	177–415	177–415
	1–2 years	194–504	194–504
	2–4 years	177–522	177–522
	4–7 years	186–539	186–539
	7–10 years	159–522	159–522
	10–13 years	177–495	177–495
	>13 years	168–415	168–415
Ornithine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	<168	<168
	1–6 months	<115	<115
	6–12 months	<71	<71
	1–2 years	<71	<71
	2–4 years	<62	<62
	4–7 years	<62	<62
	7–10 years	<53	<53
	10–13 years	<53	<53
	>13 years	<44	<44
Phenylalanine (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	35–283	35–283
	1–6 months	62–248	62–248
	6–12 months	97–248	97–248
	1–2 years	88–274	88–274
	2–4 years	62–186	62–186
	4–7 years	53–230	53–230
	7–10 years	44–177	44–177
	10–13 years	44–150	44–150
	>13 years	18–168	18–168
Proline (μmol/g creatinine) **To convert to μmol/mmol creatinine: multiply by 0.113	0–1 month	186–1883	186–1883
	1–6 months	<1149	<1149
	6–12 months	<124	<124
	1–2 years	<115	<115
	2–4 years	<80	<80
	4–7 years	<80	<80
	7–10 years	<80	<80
	10–13 years	<80	<80
	>13 years	<80	<80

(continued)

Urinary anion gap	Age range	Male	Female
Serine (µmol/g creatinine) **To convert to µmol/mmol creatinine: multiply by 0.113	0–1 month	707–2493	707–2493
	1–6 months	371–1715	371–1715
	6–12 months	442–1211	442–1211
	1–2 years	398–1096	398–1096
	2–4 years	283–831	283–831
	4–7 years	336–822	336–822
	7–10 years	203–610	203–610
	10–13 years	203–592	203–592
	>13 years	186–442	186–442
Taurine (µmol/g creatinine) **To convert to µmol/mmol creatinine: multiply by 0.113	0–1 month	71–1998	71–1998
	1–6 months	53–787	53–787
	6–12 months	80–1087	80–1087
	1–2 years	106–1406	106–1406
	2–4 years	115–1768	115–1768
	4–7 years	150–2033	150–2033
	7–10 years	159–2033	159–2033
	10–13 years	159–2033	159–2033
	>13 years	141–1591	141–1591
Threonine (µmol/g creatinine) **To convert to µmol/mmol creatinine: multiply by 0.113	0–1 month	177–1220	177–1220
	1–6 months	150–813	150–813
	6–12 months	124–495	124–495
	1–2 years	133–548	133–548
	2–4 years	88–424	88–424
	4–7 years	80–318	80–318
	7–10 years	80–239	80–239
	10–13 years	71–248	71–248
	>13 years	62–256	62–256
Tyrosine (µmol/g creatinine) **To convert to µmol/mmol creatinine: multiply by 0.113	0–1 month	53–486	53–486
	1–6 months	106–460	106–460
	6–12 months	97–477	97–477
	1–2 years	115–424	115–424
	2–4 years	88–265	88–265
	4–7 years	80–309	80–309
	7–10 years	62–230	62–230
	10–13 years	53–221	53–221
	>13 years	18–203	18–203
Valine (µmol/g creatinine) **To convert to µmol/mmol creatinine: multiply by 0.113	0–1 month	27–230	27–230
	1–6 months	35–168	35–168
	6–12 months	53–168	53–168
	1–2 years	62–186	62–186
	2–4 years	53–177	53–177
	4–7 years	27–133	27–133
	7–10 years	27–133	27–133
	10–13 years	27–150	27–150
	>13 years	27–115	27–115

Urinary amino acids [68]: Reference ranges provided above are based on those reported by Parvy et al. However, normative ranges vary significantly among laboratory assays. Please consult with performing laboratory for reference ranges

(continued)

Urinary anion gap	Age range	Male	Female
Urinary catecholamines			
Dopamine (mcg/24 h) **To convert to nmol/day: multiply by 6.53	<1 year	<86	<86
	1 year	10–140	10–140
	2–3 years	40–260	40–260
	≥4 years	65–400	65–400
Epinephrine (mcg/24 h) **To convert to nmol/day: multiply by 5.46	<1 year	<2.6	<2.6
	1 year	<3.6	<3.6
	2–3 years	<6.1	<6.1
	4–9 years	0.2–10	0.2–10
	10–15 years	0.5–20.0	0.5–20.0
	≥16 years	<21	<21
Metanephrine (mcg/24 h) **To convert to nmol/day: multiply by 5.07	<36 months	not established	not established
	3–8 years	29–92	18–144
	9–12 years	59–188	43–122
	13–17 years	69–221	33–185
	≥18 years	44–261	30–180
Norepinephrine (mcg/24 h) **To convert to nmol/day: multiply by 5.91	<1 year	<11	<11
	1 year	1–17	1–17
	2–3 years	4–29	4–29
	4–6 years	8–45	8–45
	7–9 years	13–65	13–65
	≥10 years	15–80	15–80
Normetanephrine (mcg/24 h) **To convert to nmol/day: multiply by 5.46	<36 months	not established	not established
	3–8 years	34–169	29–145
	9–12 years	84–422	55–277
	13–17 years	91–456	57–286
	18–29 years	103–390	103–390
Total metanephrine (mcg/24 h) **To convert to nmol/day: multiply by 5.26	<36 months	not established	not established
	3–8 years	47–233	57–210
	9–12 years	201–528	107–394
	13–17 years	120–603	113–414
	18–29 years	190–583	142–510
Urinary catecholamines: values are reported for Mayo Clinic Laboratories (Rochester, MN) [69, 70] individual laboratory assays may vary. Sensitivity and specificity for urine catecholamines differ based on cutoff used. To increase specificity, higher cutoffs than the established reference ranges in healthy populations have been used: norepinephrine >170 mcg/24 h, epinephrine >35 mcg/24 h, dopamine >700 mcg/24 h, normetanephrine >900 mg/24 h, and metanephrine >400 mcg/24 h, total metanephrines >1300 mg/24 h Using combined 24 h metanephrines and catecholamines (positive test defined as either urine metanephrines or any catecholamine is elevated) has resulted in a sensitive and specificity of 98% [71, 72]			
Urine creatinine excretion, pH, and maximum osmolality			
Maximum osmolality (mOsm/L) [45]	10–30 days	775–963	775–963
	1–2 months	963–1051	963–1051
	2–6 months	1051–1156	1051–1156
	6 months to 1 year	1156–1206	1156–1206
	1–5 years	1206–1285	1206–1285
	5–18 years	1308–1324	1308–1324

(continued)

Urinary anion gap	Age range	Male	Female
Urine creatinine excretion (mg/kg/day) [73] **To convert to mmol/kg/day: multiply by 0.00884	3 years	10.9–21.4	8.9–20.6
	4–5 years	12–24.2	12.3–21.2
	6–8 years	15.2–25.6	12.4–26.6
	9–13 years	11.3–27.7	13.2–27.6
	14–18 years	13.2–33.3	14.6–26.9
Maximum concentration ability based on the regression line $Y = 1400 \times (1–0.943*t^{-0.325})$, where t = days. Standard deviation for any time point was 159			
Urine pH [74]	2–7 years	5.71–7.16	5.69–7.3
	7–13 years	5.69–7.01	5.71–7.00
	13–18 years	5.63–6.90	5.66–6.96
Urine electrolytes and metabolic risk factors for nephrolithiasis			
24-h urine calcium [75]	1–18 years	<4 mg/kg/24 h (<0.1 mmol/kg/24 h)	<4 mg/kg/24 h (<0.1 mmol/kg/24 h)
Urine calcium/creatinine ratio (mg/mg) [76] **To convert to SI units (mmol/mmol): multiply by 2.82	1 month to 1 year	0.81	0.81
	1–2 years	0.56	0.56
	2–3 years	0.5	0.5
	3–5 years	0.41	0.41
	5–7 years	0.3	0.3
	7–10 years	0.25	0.25
	10–14 years	0.24	0.24
	14–17 years	0.24	0.24
24-h urine phosphorus (mg/kg/day) [77] **To convert to mmol/kg/day: divide by 30.97	2–3 years	11.1–30.7	10.8–34.1
	3–4 years	10.8–29.7	10.5–34.1
	4–5 years	10.2–29.1	9.9–31.0
	5–6 years	9.9–28.2	9.6–31.0
	6–7 years	9.3–27.3	9.3–29.4
	7–8 years	9.0–26.6	8.7–27.9
	8–9 years	8.4–25.7	8.4–26.6
	9–10 years	8.1–25.1	7.7–25.1
	10–11 years	7.7–24.2	7.4–23.8
	11–12 years	7.1–23.2	6.8–22.3
	12–13 years	6.8–22.6	6.5–21.1
	13–14 years	6.2–21.7	6.2–19.5
	14–15 years	5.9–21.1	5.6–18.3
	15–16 years	5.3–20.1	5.3–16.7
	16–17 years	5.0–19.2	4.6–15.5
	17–18 years	4.6–18.6	4.3–13.9
Urine phosphorus/creatinine ratio (mg/mg) [76] **To convert to SI units (mmol/mmol): multiply by 3.65	1 month to 1 year	0.34–5.24	0.34–5.24
	1–2 years	0.34–3.95	0.34–3.95
	2–3 years	0.34–3.13	0.34–3.13
	3–5 years	0.33–2.17	0.33–2.17
	5–7 years	0.33–1.49	0.33–1.49
	7–10 years	0.32–0.97	0.32–0.97
	10–14 years	0.22–0.86	0.22–0.86
	14–17 years	0.21–0.75	0.21–0.75

(continued)

Urinary anion gap	Age range	Male	Female
24-h urine citrate (mg/day) [74] **To convert to mmol/day: multiply by 0.005205	2–7 years	67–659	87–734
	7–13 years	102–989	162–1067
	13–18 years	130–1328	231–1589
Urine citrate/creatinine ratio (mg/g creatinine) [74] **To convert to mmol/mol: multiply by 0.589	2–7 years	243–1778	290–2370
	7–13 years	139–1212	261–1462
	13–18 years	87–976	216–1404
Calcium to citrate ratio (mg/mg) [74] **To convert to mmol/mmol: multiply by 4.79	2–7 years	0.05–0.48	0.03–0.42
	7–13 years	0.05–0.60	0.04–0.47
	13–18 years	0.06–0.80	0.05–0.60
Assessment of nephrolithiasis [74, 78]: hypocitraturia has usually been defined as a citrate/creatinine ratio (mg/g) of <180. Cutoff values normalized to body surface area are less age dependent, and values of 180 mg/1.73m^2/day (males) and 250 mg/1.73m^2/day (females) have been suggested. The calcium to citrate ratio has also been used in the assessment of nephrolithiasis, with a value of 0.326 providing good discrimination between normal controls and children with nephrolithiasis			
24-h urine magnesium (mg/kg/day) [79] **To convert to mmol/kg/day: multiply by 0.0411	3–5 years	1.1–3.8	1.1–3.8
	6–8 years	1.0–4.0	1.0–4.0
	9–11 years	0.4–3.4	0.4–3.4
	12–14 years	0.4–3.3	0.4–3.3
	15–16 years	0.5–3.0	0.5–3.0
Urine magnesium/creatinine ratio (mg/mg) [76] **To convert to mmol/mmol: multiply by 4.65	1 month to 1 year	0.10–0.48	0.10–0.48
	1–2 years	0.09–0.37	0.09–0.37
	2–3 years	0.07–0.34	0.07–0.34
	3–5 years	0.07–0.29	0.07–0.29
	5–7 years	0.06–0.21	0.06–0.21
	7–10 years	0.05–0.18	0.05–0.18
	10–14 years	0.05–0.15	0.05–0.15
	14–17 years	0.05–0.13	0.05–0.13
Oxalate (24-h collection) [80]	all ages	<45 mg/1.73m^2/day (<0.50 mmol/1.73m^2/day	<45 mg/1.73m^2/day (<0.50 mmol/1.73m^2/day
Urine oxalate/creatinine ratio (mg/mg) [81] **To convert to mmol/mmol: multiply by 1.255	1 month to 6 months	0.056–0.175	0.056–0.175
	6 months to1 year	0.048–0.139	0.048–0.139
	1–2 years	0.040–0.103	0.040–0.103
	2–3 years	0.032–0.080	0.032–0.080
	3–5 years	0.024–0.064	0.024–0.064
	5–7 years	0.024–0.056	0.024–0.056
	7–17 years	0.016–0.048	0.016–0.048
Hyperoxaluria: There is no clear cutoff for primary versus secondary hyperoxaluria. Urinary excretion greater than 0.7 mmol (63 mg)/1.73m^2/day is more likely to have a metabolic cause. Reference ranges may vary based on laboratory assay used			
Urinary glycolate/creatinine ratio (mg/g) [80] **To convert to mmol/mol: multiply by 1.507	0–6 months	<241–282	<241–282
	7–24 months	<163–194	<163–194
	2–5 years	<127–152	<127–152
	5–14 years	<110–123	<110–123
	>16 years	<66–83	<66–83

(continued)

Urinary anion gap	Age range	Male	Female
Urinary L-glycerate-to-creatinine ratio (mg/g) [80] **To convert to mmol/mol: multiply by 1.066	0–6 months	<13–193	<13–193
	7–24 months	<13–193	<13–193
	2–5 years	<13–193	<13–193
	5–14 years	<22–129	<22–129
	>16 years	<129	<129
Increased concentrations of oxalate and glycolate indicate type 1 hyperoxaluria; increased concentrations of oxalate and glycerate indicate type 2 hyperoxaluria. Reference ranges may vary based on performing laboratory			
Uric acid (24-h collection) [82]	>2 years	<815 mg/1.73m^2/day (<4.85 mmol/1.73m^2/day)	<815 mg/1.73m^2/day (<4.85 mmol/1.73m^2/day)
Urine uric acid/creatinine ratio (mg/mg) [80] **To convert to mmol/mmol: multiply by 0.673	1 month to 6 months	1.189–2.379	1.189–2.379
	6 months to 1 year	1.040–2.229	1.040–2.229
	1–2 years	0.743–2.080	0.743–2.080
	2–3 years	0.689–1.932	0.689–1.932
	3–5 years	0.594–1.635	0.594–1.635
	5–7 years	0.446–1.189	0.446–1.189
	7–10 years	0.386–0.832	0.386–0.832
	10–14 years	0.297–0.654	0.297–0.654
	14–17 years	0.297–0.594	0.297–0.594
½ Cystine µmol/g creatinine [68] (reported as ½ Cystine because initial methods of quantification involved a reduction of cystine followed by an assay for cysteine) **To convert to µmol/mmol creatinine: multiply by factor of 0.113	0–1 month	212–690	212–690
	1–6 months	115–424	115–424
	6–12 months	106–256	106–256
	1–2 years	88–230	88–230
	2–4 years	71–265	71–265
	4–7 years	71–194	71–194
	7–10 years	71–186	71–186
	10–13 years	62–203	62–203
	>13 years	53–301	53–301
Cystine µmol/g creatinine (reported as cystine) [83] **To convert to µmol/mmol creatinine: multiply by factor of 0.113	< or = 12 months	12–504	12–504
	13–35 months	11–133	11–133
	3–6 years	<130	<130
	7–8 years	<56	<56
	9–17 years	<104	<104
	> or = 18 years	10–98	10–98
Cystinuria [84]: Assays may vary between laboratories. Children with cystinuria generally have markedly elevated urinary cystine levels, with values >1300 µmol/g, but the amount can vary markedly with some having values exceeding 3000. More mild elevations can be seen in asymptomatic heterozygote carriers of type B cystinuria			
Urine microscopy			
Cells (RBC/hpf) [85, 86]	Birth to 18 years	0–4 RBC/HPF (centrifuged)	0–4 RBC/HPF (centrifuged)
	Birth to 18 years	0–5 RBC/mm^3 (uncentrifuged)	0–5 RBC/mm^3 (uncentrifuged)
Cells (WBC/hpf) [87]	Birth to 18 years	0–4 WBC/HFP (centrifuged)	0–4 WBC/HFP (centrifuged)
	Birth to 18 years	0–9 WBC/mm^3 (uncentrifuged)	0–9 WBC/mm^3 (uncentrifuged)

(continued)

Urinary anion gap	Age range	Male	Female
Urine protein			
B2-microglobulin/creatinine ratio (μg/g) [88, 89]	all ages (random)	≤300 μg/g	<300 μg/g
Microalbuminuria (mg albumin/ gm creatinine) [90, 91]	10–18 years	30 mg/g	30 mg/g
Spot urine protein/creatinine ratio (mg/mg) [90, 92]	6–24 months	<0.5	<0.5
	>2 years	<0.2	<0.2
Urinary protein (24-h urine collection) [90]	1–18 years	<100 mg/m^2/day	<100 mg/m^2/day
Tubular function assessment			
Magnesium fractional excretion (FeMg) (%) [93]	All ages	0.5–4	0.5–4
Magnesium fractional excretion [94]: FeMg formula: [(Mg urine × Scr)/(0.7 × Mg plasma × Cr urine)] × 100 The factor of 0.7 adjusts total plasma Mg concentration to the freely filtered fraction A FeMg >4% is consistent with renal Mg wasting, while a FeMg <2% is consistent with an extrarenal origin of hypomagnesemia. The evaluation has to be made with normal Mg serum values			
Tubular maximum reabsortion for phosphate corrected for GFR (TmP/GFR) (mmol/l) [95]	2 to <4 years	1.04–2.79	1.04–2.79
	4 to <6 years	1.05–2.60	1.05–2.60
	6 to <8 years	1.26–2.35	1.26–2.35
	8 to <9 years	1.1–2.31	1.1–2.31
	10 to <12 years	1.15–2.58	1.15–2.58
	12 to <15 years	1.18–2.09	1.18–2.09
Tubular maximum reabsortion for phosphate corrected for GFR [96] [97]: TmP/GFR formula = {1-[(Urine p × Serum cr)/Urine cr × Serum p)]} × Serum p (where p: phosphate, cr is creatinine) In children, TmP/GFR is identical to formula TP/GFR = [Sp – (Up × Scr)/Ucr] which represents tubular phosphate reabsortion under fasting or nonfasting conditions without phosphate load			
Fractional excretion of sodium (FeNa) [98]	All ages	<1%	<1%
Fractional excretion of sodium [98]: Formula for FeNa is as follows: FeNa = [(Na urine x Scr) × 100]/(Na plasma × Cr urine) A FeNa <1% is suggestive of prerenal disease, whereas a value >2% indicates acute tubular necrosis. However, FeNa may perform poorly in several disease states, including glomerulonephritis, urinary obstruction, and nonoliguric ATN			
Fractional excretion of urea [98]	All ages	<35%	<35%
Formula for fractional excretion of urea (FeUrea) [98]: FeUrea = (Urea urine × Scr)/(Urea plasma × Cr urine) A FeUrea <35% is suggestive of prerenal disease, whereas a FeUrea >50% is indicative of AKI. However, this test may have limited utility in many patients with hospital-acquired AKI			
Transtubular potassium gradient [99]	All ages (in hyperkalemic state)	>6	>6
	All ages (in hypokalemic state)	<2	<2
Formula for transtubular potassium gradient (TTKG) [99]: TTKG = (K urine × Osm blood)/(K plasma × Osm urine) Generally, a TTGK of <6 is considered indicated of impaired K secretion during hyperkalemia. Conversely, a TTKG >2 is considered inappropriate during hypokalemia. TTKG requires urine Na to be >25 meq/L and a urine osmolality that is equal to or greater than the serum osmolality			

[*] Normal ranges values are given as International System (SI) units. For conversion to Conventional (CON) units, please refer to online conversion resources [10]

[**] Conventional units are reported as reference ranges, given their most frequent clinical use. Conversion factors to SI units are included for each analyte

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